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INDEX OF SUBJECTS.

	PAGE
A.	
Actinomyces. —A study of . . . cultivated from commercial vaccine virus - - - - -	493
Agglutinating. —A study of the . . . , hemolytic, and endo- theliolytic action of the blood serum in variola - - - - -	157
Agglutination of the pneumococcus with certain normal and im- mune sera - - - - -	228
Agglutinines. —The non-identity of . . . acting upon the flagella, and upon the body of bacteria - - - - -	89
Ammonia. —On the output of . . . in the course of different forms of insanity - - - - -	449
Autolysis. —On the . . . of brain tissue - - - - -	212
B.	
Bactericidal action of the blood serum in variola and varioloid -	196
Bacterium pyogenes sanguinarium - - - - -	402
Blood. —Observations on the coagulation time of the blood and the blood plates - - - - -	120
Blood. —Cat's . . . , differential counts of the leucocytes -	250
Blood. —The reactions of the . . . in experimental diabetes mellitus - - - - -	255
Blood. —The influence of certain bacteria on the coagulation of the . . . - - - - -	407
Blood. —The shape of the mammalian red blood corpuscles -	513
Brain. —On the autolysis of . . . tissue - - - - -	204
C.	
Chinese. —Observations, especially with the Röntgen rays on the artificially deformed foot of a . . . lady of rank, in relation to the functional pathogenesis of deformity - - - - -	420
Chromatin. —On the chemistry of the . . . substance in the nerve cell - - - - -	204
Coagulation. —The influence of certain bacteria on the . . . of the blood - - - - -	407
Colon bacillus. —The occurrence of the . . . on the hands -	463
Connective tissue. —A hitherto undescribed fibrillar substance produced by the . . . cells - - - - -	334

	PAGE
D.	
Delhi sore. —Protozoa in a case of tropical ulcer	472
Diabetes. —The reactions of the blood in experimental . . . mellitus	255
Digestion. —On the . . . and self-digestion of tissues and tissue extracts	217
Dysentery. —The . . . bacillus in a series of cases of infantile diarrhea	11
Dysentery. —The reaction of certain water bacteria with . . . immune serum	21
E.	
Endothelolytic. —A study of the agglutinating, hemolytic, and . . . action of the blood serum in variola	157
Erythrocytes. —On the appearance and significance of certain granules in the . . . of man	342
Erythrocytes. —A study of the Volume Index	367
F.	
Fibrillar. —A hitherto undescribed . . . substance produced by the connective-tissue cells	334
Flagella. —The non-identity of agglutinins acting upon the . . . and upon the body of bacteria	89
Fluorine. —The pathology of chronic . . . poisoning . . .	301
Forage poisoning. —A pathology for . . . or the so-called epizootic cerebro-spinal meningitis of horses	243
G.	
Granules. —On the appearance and significance of certain . . . in the erythrocytes of man	342
H.	
Hands. —The occurrence of the colon bacillus on the	463
Hemolysis. —The relations of specific gravity and osmotic pressure to	1
Hemolysins. —A study of the proteolytic enzymes and of the so- called . . . of some of the common saprophytic bacteria . .	42
Hemolytic. —The connection between the alkalinity of certain bacterial filtrate and their . . . power	31
Hemolytic. —A study on the agglutinating . . . and endothe- liolytic action of the blood serum in variola	157
Hip. —Resistance of the muscles in reducing a congenitally dis- located	437
Hips. —Mechanism for reducing congenitally dislocated	440
Hip. —Methods of treatment of congenital dislocation of the . . .	433

I.

Inflammation. — On a difference in the influence upon . . .	
between the section of the sympathetic nerve and the removal	
of the sympathetic ganglion	135
Insanity. — On the output of ammonia in the course of different	
forms of	449
Intestinal wall. — The passage of tubercle bacilli through the	
normal	460

L.

Lead. — Pathological changes in the nerve system in a case of	
. . . poisoning	142
Leucocytes. — Cat's blood— differential counts of the	250

M.

Meningitis. — A pathology for forage poisoning, or the so-called	
epizootic cerebro-spinal meningitis of horses	243
Mercurial diuresis	132
Milk. — The physical chemistry of	127
Mucin. — As a bacterial product	101

P.

Pneumococcus. — On the agglutination of the with cer-	
tain normal and immune sera	228
Pneumonic. — The fats of exudation	109
Protozoa. — in a case of tropical ulcer ("Delhi sore") .	472
Protozoon-like. — Scarlet fever, bodies found in four cases,	483

S.

Scarlet fever. — Protozoon-like bodies found in four cases . . .	483
Streptococcus pyogenes in variola	180

T.

Thoracic. — On an abnormal duct	153
Tropical ulcer. — Protozoa in a case of ("Delhi sore") .	472
Tubercle bacilli. — The passage of through the normal	
intestinal wall	460
Tuberculosis. — Observations on the morphology of Bacillus . . .	
from human and bovine sources	313

V.

Vaccinated. — An experimental study of the bacteriolytic comple-	
ment content of the blood serum in normal, vaccinated, and	
variolated rabbits	63

	PAGE
Vaccine virus. — A study of actinomyces cultivated from commercial	493
Variola. The bacteriolytic complement content of the blood serum in	71
Variola. — A study of the agglutinating, hemolytic, and endothelialytic action of the blood serum in	157
Variola. — Studies in the etiology and pathology of	163
Variola. — Bactericidal action of the blood serum in and varioloid	196
Variola. — Streptococcus pyogenes in	180
Volume Index. — A study of the	367

INDEX OF NAMES.

	PAGE
A.	
Abbott, A. C.	42
B.	
Bartlett, R. W.	440
Bergey, D. H.	21
Berry, N. L., Jr.	402
Bradford, E. H.	435, 437
Brown, P. E.	420
Busch, F. C.	250
Butler, C. S.	153
C.	
Capps, J. A.	367
Christian, H. A.	109
E.	
Ernst, H. C.	313, 402
G.	
Gildersleeve, N.	42
H.	
Howard, W. T., Jr.	157, 493
J.	
Jordan, E. O.	31
L.	
Levene, P. A.	204, 212, 217, 449
Lewis, Frederick T.	513
Loeb, Leo	407
M.	
Mallory, F. B.	334, 483
McCarthy, D. J.	243
Meltzer, Clara J.	135
Meltzer, S. J.	135

	PAGE
P.	
Pay, G. O.	163, 180, 196
Perkins, R. G.	163, 180, 196
Pratt, J. H.	120
Q.	
Quinan, C.	1
R.	
Ravenel, M. P.	243, 460
Reagh, A. L.	89
Rettger, L. F.	101
S.	
Schwyzzer, F.	301
Smith, Theobald	89
Spiller, W. G.	142
Stookey, L. B.	212, 217, 449
Sweet, J. E.	255
T.	
Thompson, R. L.	63, 71
V.	
Van Bergen, C.	250
Vaughan, V. C., Jr.	342
Vejux-Tyrode, M.	132
W.	
Wadsworth, A.	228
Wilson, L. T.	437
Winslow, C. E. A.	463
Wolbach, S. B.	313
Wollstein, Martha	11
Wright, J. H.	472

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CONTENTS.

	PAGE
THE RELATION OF SPECIFIC GRAVITY AND OSMOTIC PRESSURE TO HEMOLYSIS. <i>Clarence Quinan</i>	I
THE DYSENTERY BACILLUS IN A SERIES OF CASES OF INFANTILE DIARRHEA. <i>Martha Wollstein</i>	II
THE REACTION OF CERTAIN WATER BACTERIA WITH DYSENTERY IMMUNE-SERUM. <i>David H. Bergey</i>	21
THE CONNECTION BETWEEN THE ALKALINITY OF CERTAIN BACTERIAL FILTRATES AND THEIR HEMOLYTIC POWER. <i>Edwin O. Jordan</i>	31
A STUDY OF THE PROTEOLYTIC ENZYMES AND OF THE SO-CALLED HEMOLYSINS OF SOME OF THE COMMON SAPROPHYTIC BACTERIA. <i>Alexander C. Abbott and N. Gildersleeve</i> . .	42
AN EXPERIMENTAL STUDY OF THE BACTERIOLYTIC COMPLEMENT CONTENT OF THE BLOOD SERUM IN NORMAL, VACCINATED, AND VARIOLATED RABBITS. <i>Ralph L. Thompson</i>	63
THE BACTERIOLYTIC COMPLEMENT CONTENT OF THE BLOOD SERUM IN VARIOLA. <i>Ralph L. Thompson</i>	71
THE NON-IDENTITY OF AGGLUTININS ACTING UPON THE FLAGELLA AND UPON THE BODY OF BACTERIA. <i>Theobald Smith and Arthur L. Reagh</i>	89
MUCIN AS A BACTERIAL PRODUCT. <i>Leo F. Rettger</i>	101
THE FATS OF PNEUMONIC EXUDATION. <i>Henry A. Christian</i>	109
OBSERVATIONS ON THE COAGULATION TIME OF THE BLOOD AND THE BLOOD PLATES. <i>Joseph H. Pratt</i>	120
THE PHYSICAL CHEMISTRY OF MILK. <i>Lawrence J. Henderson</i> .	127
MERCURIAL DIURESIS. <i>M. Vejux-Tyrode and Louis Nelson</i>	132
ON A DIFFERENCE IN THE INFLUENCE UPON INFLAMMATION BETWEEN THE SECTION OF THE SYMPATHETIC NERVE AND THE REMOVAL OF THE SYMPATHETIC GANGLION. <i>S. J. Meltzer and Clara J. Meltzer</i>	135
THE PATHOLOGICAL CHANGES IN THE NERVE SYSTEM IN A CASE OF LEAD POISONING. <i>William G. Spiller</i>	142
ON AN ABNORMAL THORACIC DUCT. <i>Charles S. Butler</i>	153

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VOL. X.

AUGUST, 1903.

No. 1.

THE RELATIONS OF SPECIFIC GRAVITY AND OSMOTIC PRES-
SURE TO HEMOLYSIS.¹

CLARENCE QUINAN.

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The fact is well established that if an animal of a given species be injected with the blood corpuscles of an individual of different species the tissues of the host react to the intruding substance and acquire after some time a peculiar and specific character. The clear blood serum of such an animal affects in a remarkable way the blood corpuscles of that species whose blood was selected for the immunizing injections, dissolving them freely within certain limits of dilution and temperature.

Two substances of uncertain identity are considered to be indispensable elements in the equation of globulysis: one, thermostabile, and known variously as amboceptor or sensibilisator; the other, thermolabile, with a thermal death point between 50–65° C., usually termed complement or alexin. These bodies are believed to react in obedience to the well-known lateral chain theory of Ehrlich, which appears to bring in harmony the greatest number of experimental facts, without making quite clear the absolute mode of action.

The phenomenon of globulysis has several characteristics that serve to differentiate it from ordinary chemical reactions. The atomic movement here offers certain apparent anomalies; thus a temperature close to that of the body (30–45° C.) is most favorable—recalling the action of ferments, and revealing at once that however profound the atomic interchange, it is accompanied by a very insignificant energy disturbance. Again, the reaction is apparently not quantitative

¹ Received for publication May 25, 1903.

in the strict sense, though it recalls to some extent the action of N_2O_4 in the preparation of sulphuric acid by the chamber process. Finally, any attempt to identify amboceptor or complement with a definite serum group discloses the fact that however active the whole serum, its organic fractions, though prepared with the utmost circumspection with regard to temperature and asepsis, are either inert or exhibit greatly impaired activity, the specific substance often adhering to a whole series of precipitates.

Absolutely nothing is known of the chemical constitution of either of these bodies, though they are believed to be affiliated in some way with an illy defined group of globulins separable by fractional precipitation from active sera. According to some observers the serum complements are multiple, a large number of isomers being possible. In the present state of blood chemistry, however, bearing in mind the uncertainty and confusion that prevails as to the classification of the protein bodies, it is perhaps as well to await the outcome of quantitative analyses before introducing new sources of conjecture.

Disruption of erythrocytes may be induced by purely physical means, but this action is invariable and not specific; thus under certain conditions of unequal osmotic tension, as, for example, when corpuscles are introduced into nonisotonic solutions, hemoglobin exosmosis promptly occurs. This may be very readily demonstrated and goes to prove that the integrity of a specific erythrocyte depends in part upon the molecular concentration obtaining upon either side of the cell membrane. In this connection it is interesting to note that active goat serum dialyzed until it is almost salt free causes instantaneous cell solution when brought in contact with red corpuscles, the process when observed microscopically resembling that induced by distilled water. However, as will be seen later, the cell may retain its contents in spite of a considerable range of variation in the specific gravity of the surrounding serum.

Hamburger, quoting de Vries' studies of the cell properties of *Tradescantia discolor*, *Curcuma rubricaulis*, and *Begonia*

manicata, states: "Die eigentliche Zellhaut sowohl für Wasser als auch für Salze durchlässig ist, während die protoplasmaschicht, welche den Zellinhalt umgiebt (der Protoplast) semi-permeabel ist, das heisst; wohl dem Wasser nicht aber den krystalloiden Substanzen den Durchgang gestattet."

Later, reviewing his own work on the isotonic coefficients of erythrocytes, Hamburger declares that this cell exhibits virtually the same physical properties.

H. Koeppé, after a thorough research, arrived at the conclusion that blood corpuscles are not permeable for salts as such, but solely for ions, and so far as the alkali salts are concerned, only for their anions.

This view is shared by Gurber, who has shown that if CO_2 be passed through a suspension of corpuscles in NaCl solution, the liquid acquires an alkaline reaction. Exact quantitative analyses proved that no KHCO_3 or NaHCO_3 passed out of the cells; on the contrary the K and Na figure remained constant. He therefore assigned the increased alkalinity to the mass action of sodium carbonate and hydrochloric acid, according to the equation $2 \text{NaCl} + \text{CO}_2 + \text{H}_2\text{O} = \text{Na}_2\text{CO}_3 + 2 \text{HCl}$; this evidence led him to the conclusion that the corpuscle excludes the kathions of these salts.

The direct bearing of this work upon the problems of hemolysis is at once evident, and though it fails to make clear the rationale of specific cell solution, there can be no doubt of its significance relative to the intimate structure of the erythrocyte. This is especially important because of the growing tendency to consider this cell as a chemical unit, notwithstanding the manifest absence of molecular continuity. While it is undoubtedly true that for any erythrocyte a salt solution may be prepared in which the cell will retain its normal contour — such solution being isotonic with the serum or plasma — it is a grave error to assume that .85 per cent NaCl solution is uniformly isotonic. The .5 per cent suspension of erythrocytes in this solution usually employed in hemolytic experiments, though possessing the advantage of uniformity, is open to criticism on both chemical and

physical grounds. Test cells in such a solution cannot be regarded as normal, since they are at times in unstable equilibrium.

If it could be shown that the reaction of globulysis is exothermic or endothermic, the problem would at once assume a more definite phase; even though the energy developed were not assigned as positive or negative heat of formation, at least the nature of the phenomenon would be firmly established.

If complement and amboceptor are protein bodies, then it is clear they arise either by cleavage of the serum and corpuscle proteins, or are purely additive products and enter as radicals into the structure of the protein molecule, increasing its mass. In this connection it may be stated that serum dialyzed until it is free of salts exhibits unimpaired activity if restored to its original specific gravity with sodium chloride.

This certainly indicates that hemolysis is independent of the crystalline components of the serum, or at least may occur in the absence of the greater number of those normally present.

The principal intracellular protein body is hemoglobin. It has, according to Hufner, a molecular weight of 14,129 and the formula $C_{636}H_{1025}N_{164}FeS_3O_{181}$. Taking albumen as typical of the extracellular proteins, again a great molecule is encountered. Koeppe calculates a molecular weight of 8,848. The structural formula is of course unknown. The properties of these great atom groups are undetermined; many conflicting views obtain as to the products of decomposition of the simplest known protein, and this need occasion no surprise in view of the great technical difficulties that present themselves. There is reason to believe that the serum proteins occur in ionic combination; at least the physical data now available support such a view.

Weighing carefully the preceding facts, it becomes apparent that the identity of complement and amboceptor is merged with that of these huge passive molecules; and there is every likelihood that before the problems of globulysis are

solved, the loose inferential method of the biologist, which has disclosed this field of rich promise, must give place to the more trustworthy procedures of the chemist and physicist.

In the course of a somewhat extended study of the physical properties of hemolytic sera it was discovered that specific gravity is progressively lowered, either by the formation of specific bodies during the process of immunization or as the result of the slight periodic depletion attending the withdrawal of material for study.

This led to an investigation having a twofold object in view — the determination of the lower limit of specific gravity depression, and the corresponding variation, if any, in the reduction of the freezing point (δ). At the same time the globulytic activity of each specimen was studied and the reaction velocity at 15° C. expressed in minutes.

Immune serum was supplied by six goats that received from time to time large injections of defibrinized rabbit's blood. The necessary material was obtained from these animals by opening a small vein and extracting from twenty-five to thirty cubic centimeters of blood. This was then allowed to clot, and the clear serum removed for examination.

A suspension of rabbit erythrocytes in their own serum was used throughout this work in preference to any artificial substitute, though an almost perfect isotonic solution may be prepared in the following manner: If a known weight and volume of serum be dialyzed until free of salts, and these are then recovered by evaporating the contents of the dialyzer, the residue when dried to constant weight will be found to vary between .52 and .65 of a gramme in 100 grammes of serum. The proper salts of the serum prepared in this way yield a solution that is of course far superior to the so-called physiological solution of the single salt NaCl, but a time-consuming procedure must be resorted to for each individual, and, moreover, variations in specific gravity occur in injected animals and occasion a corresponding variation in this equivalent.

The specific gravity of the blood serum of normal Belgian

hares varies little from 1.0250 with a value for (δ) of .63. Repeated determinations showing little individual variation, these figures were accepted as normal.

The reaction was observed in the following way: Two parts of hemolytic serum and three parts of the standard suspension having been measured out with the pipette of the Thoma-Zeiss Hemocytometer, the mixture was brought into the counting chamber of the same apparatus, and the lapse of time noted until globulysis was complete.

Extreme precautions were taken to insure the accuracy of the specific gravity determinations. Picnometers of five grammes capacity, and U tubes having long capillary tips were employed. The apparatus was graduated with the utmost care, and all weighings were done with the Sartorius short arm analytical balance.

The depression of the freezing point was observed with Beckman's apparatus, somewhat modified to admit the use of the small amount of serum available for each determination. Under the best conditions the inherent error of this instrument is considerable, and there can be no doubt that it is much exaggerated by any change in the volume of the inner chamber.

The simple formula of Nernst and Abegg $T_0 = t' + \frac{k}{K} (t' - t_0)$ will serve to make this clear. In this T_0 is the true temperature and t' the observed; k and K are constants, and t_0 the convergence temperature. To obtain T_0 , then, it is necessary to apply the correction $\frac{k}{K} (t' - t_0)$ to the observed temperature t' .

Two opposed factors affect a liquid whose temperature is being reduced in this apparatus; the freezing mixture, which tends to restrict motion, and the stirring device which has the reverse effect. From these two influences a temperature results, known as the convergence temperature t_0 . Its value depends upon the specific heat, size, form, and heat conductivity of the inner chamber and its contents, as well as upon the energy developed by stirring and the frictional resistance overcome. Neglecting other sources of error, the value of t_0 in this series was necessarily large. For this

reason no great precision can be claimed for these figures, but taken with the exact specific gravity determinations they possess much comparative interest. Fairly concordant results were obtained by making a long series of observations on each specimen. The readings showed a maximum variation of -0.03° .

TABLES I-VI.

Showing the comparative physical characteristics of the reacting sera, and their relation to the rate of globulysis.

I.

DATE.	Goat (I.) Spec. grav. of serum. 15. Cent.	Goat (I.) ($\delta =$)	Spec. grav. of rabbit serum. 15. Cent.	Rabbit ($\delta =$)	Globulysis complete (minutes) 15. Cent.
1902					
March 19	1.0336	— .58	1.0250	— .63	15''
" 22	1.0325	— .59	"	"	16''
" 27	1.0324	— .61	"	"	9''
" 30	1.0326	— .61	"	"	9''
April 2	1.0299	— .72	"	"	9''
" 5	1.0311	— .72	"	"	9''
" 10	1.0319	"	"	8''
1903					
March 19	1.0313				

II.

DATE.	Goat (II.) Spec. grav. of serum 15. Cent.	Goat (II.) (δ)	Spec. grav. of rabbit serum 15. Cent.	Rabbit ($\delta =$)	Globulysis complete (minutes) 15. Cent.
1902					
March 19	1.0270	— .71	1.0250	— .63	18"
" 22	1.0261	— .56	"	"	17"
" 27	1.0255	— .66	"	"	8"
" 30	1.0241	— .63	"	"	8"
April 2	1.0222	— .59	"	"	8"
" 5	1.0232	— .65	"	"	8'
" 10	1.0222	"	"	10"
1903					
February 17.....	1.0232				

III.

DATE.	Goat (IV.) Spec. grav. of serum 15. Cent.	Goat (IV.) ($\delta =$)	Spec. grav. of rabbit serum 15. Cent.	Rabbit ($\delta =$)	Globulysis complete (minutes) 15. Cent.
1902					
March 21	1.0269	— .57	1.0250	— .63	—
" 26	1.0250	— .58	"	"	11"
" 29	1.0263	— .64	"	"	9"
April 1	1.0244	— .65	"	"	10"
" 4	1.0232	— .72	"	"	10"
" 9	1.0239	— .72	"	"	12"
1903					
March 2	1.0232				

IV.

DATE.	Goat (V.) Spec. grav. of serum 15. Cent.	Goat (V.) ($\delta =$)	Spec. grav. of rabbit serum 15. Cent.	Rabbit ($\delta =$)	Globulysis complete (minutes) 15. Cent.
1902					
March 20	1.0302	— .68	1.0250	— .63	10''
“ 25	1.0263	— .53	“	“	25''
“ 28	1.0261	— .56	“	“	8''
“ 31	1.0246	— .61	“	“	7''
April 3	1.0226	— .67	“	“	8''
“ 8	1.0246	— .70	“	“	8''
“ 10	1.0250	“	“	8''

V.

DATE.	Goat (VI.) Spec. grav. of serum 15. Cent.	Goat (VI.) ($\delta =$)	Spec. grav. of rabbit serum 15. Cent.	Rabbit ($\delta =$)	Globulysis complete (minutes) 15. Cent.
1902					
March 20	1.0303	— .68	1.0250	— .63	8''
“ 25	1.0284	— .55	“	“	10''
“ 28	1.0279	— .61	“	“	10''
“ 31	1.0279	— .62	“	“	8''
April 3	1.0268	— .73	“	“	8''
“ 8	1.0259	— .72	“	“	8''
“ 10	1.0250	—	“	“	8''

VI.

DATE.	Goat (VII.) Spec. grav. of serum 15. Cent.	Goat (VII.) ($\delta =$)	Spec. grav. of rabbit serum 15. Cent.	Rabbit ($\delta =$)	Globulysis complete (minutes) 15. Cent.
March 21	1.0269	— .63	1.0250	— .63	—
“ 26	1.0265	— .55	“	“	9"
“ 29	1.0263	— .62	“	“	8"
April 1	1.0255	— .62	“	“	7"
“ 4	1.0257	— .64	“	“	7"
“ 9	1.0262	— .72	“	“	10"

The data embodied in these tables are believed to justify the following conclusions:

1. If a goat be given occasional large injections of defibrinized rabbit's blood, its serum suffers a progressive loss of specific gravity.

2. The molecular concentration of the serum, as expressed by the depression of the freezing point, is correspondingly increased.

3. There is no constant relation between the specific gravity of active serum and the rate of globulysis.

4. After a definite degree of depression the specific gravity becomes constant, and a certain value is peculiar to each animal.

This study has been carried on by the aid of the Rockefeller Institute of Medical Research.

THE DYSENTERY BACILLUS IN A SERIES OF CASES OF
INFANTILE DIARRHEA.¹

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The systematic bacteriological study of the stools in cases of diarrhea in infants and young children, following the observations of Duval and Bassett,² was begun in October, 1902, with the view of determining the presence or absence of *Bacillus dysenteriae* (Shiga) in all cases of diarrhea, and whether any special group of clinical symptoms characterizes the cases in which the organism is found.

The material at the New York Foundling Hospital having been placed at the disposal of the Rockefeller Institute through the courtesy of the medical board, the cases sent to the ward for gastro-intestinal diseases were examined as they came, without selection as to previous condition or disease. The occasion to study cases occurring during the winter months was considered especially interesting, as helping to establish the identity of this class of infantile diarrheas with the more frequent cases occurring during the summer months.

One hundred and fourteen cases were studied, of which one occurred at the New York Infant Asylum, three at the Nursery and Child's Hospital, fifty at the Babies' Hospital, and sixty at the Foundling Asylum.

The technic used was that recommended by Dr. Flexner and his pupils. The stools were obtained as fresh as possible; where blood and mucus were present some was carefully taken on a sterile swab or platinum loop, and suspended in neutral broth or peptone water. From this suspension

¹ Received for publication June 14, 1903. Read before the New York Pathological Society, May 20, 1903.

² American Medicine, Sept. 13, 1902.

agar plates were poured at once or within an hour. Duval found that the dysentery bacillus grows more profusely on agar that is slightly acid in reaction, and throughout this work such a medium was used. After the plates had been in the thermostat for twenty-four hours a number of glucose agar tubes were inoculated from them, and twenty-four hours later the gas-producing organisms were rejected. From the tubes without gas, transplants were made to plain acid agar, and then the bacilli were tested as to their reaction with serum from a horse immunized with the Flexner Manila organism.

This serum was obtained first from Dr. Park, and later from Dr. Flexner. Finally the two varieties of anti-dysenteric serum sent by Dr. Flexner to the Babies' Hospital, for the treatment of dysentery patients, were used. One of these is the "Harris," the other the "Shiga" serum. Bacilli giving a positive reaction in a dilution of one to fifty were tested with both sera in dilutions up to one to three thousand.

Stools from healthy infants were examined in ten cases. The dysentery bacillus was not found. Ten newly-born infants were studied at the Lying-in Hospital, the stools being examined repeatedly from the age of twelve hours to eight days. In no instance was the dysentery bacillus present.

Conversely, no case from which the stools contained blood as well as mucus failed to show the dysentery bacillus in culture; but many of the negative cases contained large quantities of mucus and undigested food, without blood. It would seem that the tendency in cases of infection with *Bacillus dysenteriae* is toward the classical picture of dysentery, as regards the clinical symptoms. Of the one hundred and fourteen cases studied, the dysentery bacillus was found in thirty-nine. The ages of the children varied from five weeks to four years, but only twenty-one were over one year old, and seven over two. The ages of the thirty-nine positive cases were as follows:

2 months . . .	3	13 months . . .	1
3 " . . .	3	15 " . . .	2
5 " . . .	3	18 " . . .	1
6 " . . .	9	21 " . . .	1
7 " . . .	2	2 years . . .	3
8 " . . .	4	3 " . . .	1
10 " . . .	2		—
11 " . . .	1		39
12 " . . .	3		

Thus twenty-seven were under one year old, and nine were under six months, while eight were between one and two years, and four over two. Of these positive cases, twenty-nine died; one left the hospital improved, but not well; and nine were cured.

The character of the stools varied greatly. Blood appeared in twenty, usually as small specks mixed with or streaking the mucus, and not in every stool. In three cases fluid blood was present, and many stools consisted entirely of blood and mucus. In every case mucus was passed, usually in very large amounts; curds and undigested food were also frequent. The stools varied from two to nine in twenty-four hours, were in most cases green in color, and accompanied by marked tenesmus in five instances. The absence of this symptom in a large number of cases is due, in part at least, to lack of observation. In uncomplicated cases the temperature ranged from ninety-eight to one hundred and three degrees Fahrenheit, there being an initial rise; but in nine cases it never rose above one hundred and eight-tenths degrees Fahrenheit. The fatal cases ran their course in three days to six weeks, by far the greater number dying during the first or early in the second week. Only three lived four weeks or longer. Recovery took place after one to four weeks.

The bacillus isolated from these cases is a short, non-motile rod with rounded ends, sometimes almost coccoid in form, occurring singly or in pairs. It is Gram negative, and does

not liquefy gelatine nor coagulate milk. No gas is formed in glucose, saccharose, nor lactose media. On agar plates the superficial colonies are pearly white — characteristically so. Microscopically they are round, finely granular, and have regular edges which become irregular after twenty-four to forty-eight hours. The deep colonies are round or lenticular, also granular and at first regular. Agar slants are dry, irregular at the edges, cream white in color, and spread but little beyond the line of inoculation. Neutral broth becomes cloudy, and a finely granular precipitate forms. Two of my cultures form a thin pellicle after five to six days, comparable to that described by Hiss¹ in the case of his "Bacillus Y." No pellicle is developed by any of the other dysentery bacilli isolated in this series. Indol is formed after three days, but in some instances seven days elapse before the reaction appears.² One bacillus (S.A.), isolated from the second case studied at the Foundling Asylum, forms no indol at any time. This culture is the only one of the thirty-nine in my series which does not ferment mannite. In this and in its serum reactions it agrees with the Shiga and New Haven (Flexner) cultures used for control, and for which I am indebted to Dr. Park.³ All the rest form acid in the presence of mannite and maltose as well as levulose, galactose, and glucose.

The agglutination reactions are fairly uniform. All the bacilli react with the Flexner Manila ("Harris") serum in dilutions of one to fifty to one to three thousand, and with the Shiga serum up to one to two hundred only. The culture S.A., which does not ferment mannite, reacts with the Shiga horse serum in dilutions of one to three thousand, as do the Shiga and the New Haven cultures. With the Harris serum it reacts in one to five hundred, as also do the Shiga and New Haven (Flexner) bacilli.

¹ Transactions of the New York Pathological Society, January, 1903.

² Dextrose-free broth was used for indol reactions.

³ The case from which this organism came was a boy five months old, whose stools consisted entirely of blood and mucus; tenesmus was marked. He died on the third day, his blood having given a negative reaction a few hours before death. He had never been outside of New York City.

The blood reactions in these young infants are, at first sight, uncertain in their appearance; on closer study they are seen to agree with the observations made by others in adult cases. Thus they were absent altogether in twelve cases; in six others the blood could not be obtained. In the remaining twenty-one a good reaction was present as follows: on the first day in one case; third day in one case; fourth day in two cases; sixth day in two cases; seventh day in three cases; eighth day in three cases; ninth day in one case; thirteenth day in one case; only after the injection of "Harris" serum in seven, the serum having been given on the third, fourth, fifth, sixth (two cases), eighth, and fourteenth days, and the blood having been found negative before that time.

Rosenthal,¹ testing agglutinations in thirty adult dysentery patients in Moscow, found the reaction absent during the first week, strong from the tenth to the twelfth days, and diminishing during the fourth and fifth weeks, the fifty-second day being the latest time at which he found it. In several fatal cases it was weak or entirely absent. Leonard Rogers² also found it present from the sixth day on, but least marked under ten days. Of the twelve positive cases which gave no serum reaction in this series, two died on the third day; one each on the fourth, fifth, and sixth days; two on the seventh day; and two on the twelfth; one mild case recovered in one week and one case died after two weeks. So that of the twelve positive dysentery cases which gave no serum reaction, eight lasted one week and three others were over within two weeks.

The reactions were made with the bacillus obtained from the case, with that isolated from the first case examined (F.M.), and with the stock "Harris" and "Shiga" cultures. No reaction was obtained with the blood of any case and the Shiga organism.

Within twenty hours after an injection of twelve cubic centimeters of "Harris" (Flexner Manila) serum, an excellent reaction was obtained in five cases, in dilutions of one to

¹ Deutsche Med. Wochenschrift, Feb. 5, 1903.

² The Indian Medical Gazette, February, 1903.

fifty and one to one hundred. Eight days later the reaction was good in two cases in one to five hundred with the "Harris" bacillus, negative at all times with "Shiga." In one instance the reaction was complete in two hours on the twenty-seventh day after the serum had been administered, when the child was discharged. Another baby left the hospital twenty days after the inoculation, her blood giving a positive reaction in one to one hundred. Neither case had given a positive reaction before the administration of serum fourteen and eight days after the onset of diarrhea.

In one of the other cases the serum reaction was positive for a period of one week, and was not present again until death occurred two weeks later. Again it was present from the seventh day until death on the twenty-second. In another instance it was complete in one hour, in dilutions of one to five hundred, for a period of two months, when the child died, the stools having been formed and good for three weeks. In one case which recovered without serum treatment the blood gave a positive reaction for sixteen days, and in another infant for four weeks.

The bacilli were most numerous in the stools containing blood and mucus, but they were also found in those which consisted of fecal matter with only a small amount of mucus.

Rosenthal¹ found *Bacillus dysenteriae* not present in the stools later than the twenty-first day, and that in but one case. In the present series the organism was isolated from the colon at autopsy three weeks after the onset of the disease in two cases. It was not present at autopsy nineteen days after the onset in one case, and in the third week in two others. During life it had disappeared on the twenty-fourth day from the stools of one child. In two cases treated with "Harris" anti-dysenteric serum, the bacilli were not present on the fifteenth day (nine days after the injection) and on the twenty-first day (fifteen days after the injection) respectively. The stools were good. In a third case the bacilli were found in the stools twenty days after the serum had been given, and twenty-nine days after the onset of the

¹ Loc. cit.

disease. They had disappeared two days later. That they may be present for a period of two months after the onset of an attack may be inferred from the following case: Among the children examined as a matter of routine in the gastro-enteritis ward at the Foundling Asylum was a girl of ten months, in fairly good condition. Her stools were yellow, smooth and good, with a trace of mucus occasionally. From one such *Bacillus dysenteriae* was isolated. Her blood gave an excellent reaction in one to one hundred with the Flexner ("Harris") bacillus; not with the Shiga, nor with the *Bacillus coli communis*. Two months before the present observation she had had an attack of diarrhea for three weeks, with four green and mucous stools per day. No blood was passed at any time. The child went out to board, and six months later was reported to be in good condition, without intestinal disturbance during that time.

Bacillus dysenteriae was not found in the heart's blood nor in any viscus at autopsy in five cases. A septicemia due to *Bacillus dysenteriae* seems to be a rare occurrence. Rosenthal¹ found one such case among fourteen autopsies. Duval and Bassett² succeeded in cultivating the bacillus from the mesenteric glands and liver in only one case.

Infection in the hospital occurred in several instances, both at the Foundling Asylum and at the Babies' Hospital. Two are of especial interest: C. S., a female infant of ten months, convalescing from pneumonia, was well nourished, without fever, and doing well. There were three cases in the ward from which *Bacillus dysenteriae* had been isolated, when the child developed diarrhea quite suddenly, accompanied at once by marked prostration and a temperature of 103° F. The stools were small, never more than four in number, and consisted of green fecal matter, mucus, and a few specks of blood. From one of the stools on the second day *Bacillus dysenteriae* was isolated with ease, the colonies being numerous on the plates. The blood gave a negative reaction on the second and third days, positive in one to

¹ Loc. cit.

² American Medicine, Sept. 13, 1902.

fifty on the fourth and sixth. Death came on the seventh day.

The second case (J. M.) was that of a poorly developed child two years old. There was no history of any diarrhea, and she had one soft brown stool a day. At this time the stools of every case in the ward were being examined, in order to determine whether *Bacillus dysenteriae* were present in cases which gave no symptoms. Only gas-producing bacilli were isolated in her case. Two days later her stools became thin, yellow, four in number, with mucus and a little blood. *Bacillus dysenteriae* was isolated on the first day, and the child's blood gave an excellent reaction in a dilution of one to fifty. It continued positive to the day of her death two months later, in dilutions of one to five hundred, blood and mucus having disappeared from the stools three weeks before. The blood reactions were obtained with various strains of *Bacillus dysenteriae* from other cases in this series; but with the Shiga, colon, typhoid, and paratyphoid bacilli the child's blood was invariably negative in one to fifty.

In two cases of pneumonia occurring on the floor below that on which the dysentery cases were kept, the stools became frequent (seven per day) and contained much mucus, but no blood. Dysentery bacilli were present in large numbers. Death occurred three and twelve days respectively after the onset of the diarrhea. In the absence of any positive previous history it is impossible, of course, to state whether the dysentery occurred as a terminal infection, as is rational, or whether a previous infection was stimulated to an exacerbation by the intestinal irritation coincident to the attack of pneumonia.

In one case in the Babies' Hospital, a child (B. A.) with pneumonia developed loose, mucous stools, as many as nine per day. *Bacillus dysenteriae* was found, but the blood gave a negative reaction for fourteen days, when "Harris" serum was administered. The child recovered, diarrhea lasting eighteen days.

A child (J. C.) with bronchitis, from whose green and mucous stools *Bacillus dysenteriae* was readily isolated, was

discharged cured of her bronchitis, but with dysentery bacilli still in her stools ten days after admission. She was visited at her home and found to be in good condition, without any symptoms of intestinal disturbance. The significance of cases like this and B. C. as a source of infection in tenements is evident.

Four other cases occurred during convalescence from pneumonia.

A marasmic child (C. B.) acquired dysentery in the hospital, recovered from it, and is now gaining in weight. His blood gave a positive reaction during the period of four weeks.

While examining every patient in a ward in which five positive cases had been found, a two-year-old girl came under observation. She was in good condition, and the stool examined was normal. The dysentery bacillus was not found in it. The history gave no record of any diarrheal illness, but the stool was said to have been thin occasionally and to have contained a little mucus. Her blood gave a rapid and perfect reaction in thirty minutes, and during the seven weeks that she remained under observation the blood continued to react in dilutions of one to five hundred with every strain of the Manila type of the dysentery bacillus in my possession; but no agglutination was obtained with the Shiga, typhoid, colon, nor paratyphoid bacilli. There had undoubtedly been a (clinically) mild infection some time before. Repeated examinations of the stools proved negative.

To sum up, cases of infection with the dysentery bacillus in infants tend toward the clinical picture of dysentery, with frequent mucous and bloody stools.

In some cases of hospital infection, occurring as terminal to other diseases (especially pneumonia), and in other mild cases, the stools may never contain blood, but mucus is present in every case, and usually in large amount.

The serum reaction is uncertain during the first week, frequently positive after the sixth day, but may be absent for

two weeks. It cannot be relied upon for early diagnostic purposes in infants and young children.

The isolation of *Bacillus dysenteriae* from the stools is the only positive evidence of infection during life. The bacilli are present in the stools for a period of two to three weeks, but may remain for a longer time.

The type of the organism almost invariably found in these researches in New York City is that of the "Manila" or Flexner bacillus.

THE REACTION OF CERTAIN WATER BACTERIA WITH
DYSENTERY IMMUNE-SERUM.¹

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In the course of our routine bacteriological analysis of water a number of organisms were isolated and set aside for further study. The organisms in question were selected because of resemblances to the colon group. The discovery of Duval and Bassett (*American Medicine*, Vol. IV., p. 417, 1902), that the acute summer diarrheas of infants are due to *Bacillus dysenteriae*, suggested the possibility of the dissemination of this organism through polluted water.

It seemed probable that the most ready and effective method of differentiating *Bacillus dysenteriae* in the group of organisms isolated from water would be by means of the agglutination reaction with dysentery immune-serum. A supply of the immune-serum was very kindly placed at our disposal by Prof. Simon Flexner, of the University of Pennsylvania.

Among about fifty cultures set aside for further study, thirteen were found that were agglutinated with the dysentery immune-serum in 1:10 dilutions, but when these cultures were studied more carefully we were surprised to learn that each of them could readily be differentiated from *Bacillus dysenteriae* by certain of their morphological and biological characters.

After the discovery of the dissimilarity of these water bacteria to *Bacillus dysenteriae*, agglutination tests with greater dilutions of the immune-serum were instituted. We found that while most of the cultures were agglutinated in much lower dilutions than the culture of the dysentery organism employed in immunizing the horse yielding the serum, others

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reacted in relatively high dilutions. These results are shown in tabulated form in Table A.

TABLE A.
Limits of the Agglutinating Powers of Different Sera.

CULTURES.	Normal Rabbit.	Rabbit, 21/b	Rabbit, 43/a	Normal Horse.	Horse, Shiga.	Horse, New Haven.	Horse, Manila.	Normal Dog.
Dysenteria, N. Haven,	10	10	2	20	100	3,000	...	10
" Shiga . . .	10	10	2	20	100	3,000	...	10
" Pa. Hosp.	500	20	400	<10	750	175	20
" Manila . . .	75	500	20	200	50	750	250	20
Summer diarrhea, x . . .	75	100	500	400	
" " y	150	...	400	200	
Dyspeptic diarrhea, D/37	400	400	
Colitis and marasmus, M/13	150	...	500	250	
Dysenteria, G/4	60	...	150	...	500	175	
Water, 7/a	2	...	150		
" 9/b	50	...	2	...	150	...	2
" 21/b	3,000	20	2	10	250	350	10
" 43/a	2	2	200	2	10	150	70	2
" Sch/4	100	2	100	...	500	300	20
" Sch/5	100	...	2	...	2
" Sch/6	2	...	400	...	40	...	10
" Sch/b	100	100	...	20		
L/10	2	50	...	20		
L/11	2	100	20	50	...	20	...	20
L/12	2	2	100		
" 49/b	100	200	200	100	150	1,000	<500	
" 49/c	100	150	400	...	20

The results obtained demonstrate the fact that the agglutination reaction alone cannot be relied upon as a means of differentiating *Bacillus dysenteriae* from organisms which more or less closely resemble it. The agglutination test is of value as a means of differentiation when the limits of the agglutinating power of a particular serum are known. The

results obtained with two of the water organisms and a Shiga serum indicate, however, the great caution that must be observed in using this means of differentiation. The two organisms in question agglutinated in even higher dilutions of the Shiga serum than did the Shiga bacillus itself.

An explanation for the agglutinating reaction of the dysentery immune-serum with the water organisms was next sought for in the sera of normal animals. We found that the sera of normal horses, rabbits, and dogs agglutinated all of the water bacteria, as well as the different types of *Bacillus dysenteriae*, in relatively low dilutions—ranging from 1:2 to 1:400 for different organisms. The fact that the sera of normal animals contain agglutinin for such a variety of organisms has not heretofore been sufficiently emphasized. Judging from the results obtained with the serum of a rabbit immunized against one of the water organisms, it seems evident that for some of the water bacteria this was the only agglutinin present in immune-serum. On the other hand, for some of the water bacteria the agglutinins were markedly increased through the immunization process, since these reacted in much higher dilutions with the immune-serum than with the serum of a normal animal.

The serum of an animal (rabbit) immunized against one of the water bacteria showed a definite increase in its agglutinating power for some of the water bacteria, and also for the different types of *Bacillus dysenteriae*, but more especially for the Philippine group and those isolated from cases of infantile diarrhea.

In order to determine whether the agglutinins in normal and immune sera are specific, a series of absorption tests was instituted. Bouillon cultures twenty-four hours old were added to the dysentery immune-serum in proportions of 1:10 or 1:25, and placed in the incubator for two to seventy-two hours. The diluted serum was then passed through two successive sterile paper filters, when the loss of agglutinating power against the different organisms was determined. The serum of a horse immunized with the Shiga bacillus when

treated in this manner for seventy-two hours with the Shiga bacillus had lost nearly all of its agglutinating power for the Shiga or New Haven bacillus, while its agglutinating power for those of the Philippine group, the summer diarrhea organisms, and the water bacteria was reduced to a marked degree, but not nearly to the same extent as for the Shiga bacillus. When the same serum was treated in a similar manner with one of the organisms of the Philippine group it lost its agglutinating power for this group of organisms, as well as for those isolated from cases of summer diarrhea, while the agglutinating power for the water bacteria was reduced to an extent somewhat greater than that induced by the Shiga bacillus, while for the Shiga bacillus the agglutinating power was but little, if at all, affected. When the same serum was treated in a similar manner with one of the water bacteria the agglutinating power for the Shiga bacillus was but little, if at all, affected, while that for the Philippine group, the summer diarrhea organisms, and the water bacteria was reduced to some extent. These results are presented in tabulated form in Table B.

TABLE B.
Absorption Effects of Different Cultures on Normal and Immune Sera.

AGGLUTINATING CULTURES.	NORMAL HORSE.		HORSE, SHIGA.		Absorption culture.	HORSE, NEW HAVEN.		Absorption culture.	NORMAL RABBIT.		Absorption culture.	RABBIT 21/b.		Absorption culture.
	Before.	After.	Before.	After.		Before.	After.		Before.	After.		Before.	After.	
Dysentery, New Haven	20	30	N. Haven	50	N. Haven
	25	Manila	3,000	Manila
	20	—	750	750	49/b	3,000	1,000	X Sch/4	10	—	N. Haven
Dysentery, Shiga	40	N. Haven	50	N. Haven
	25	Manila	3,000	Manila
	20	—	1,000	1,000	49/b	3,000	1,500	X Sch/4
Dysentery, Pa. Hosp.	75	N. Haven
	—	Manila
	750	250	X Sch/4
Dysentery, Manila	50	50	N. Haven	75	N. Haven	20	Shiga
	—	Manila
	150	—	50	—	Manila	750	250	X Sch/4	75	—	Manila
Summer diarrhea, X	75	N. Haven	Shiga
	50	Manila
	500	50	X Sch/4	100

TABLE B. — Continued.
Absorption Effects of Different Cultures on Normal and Immune Sera.

AGGLUTINATING CULTURES.	NORMAL HORSE.		Absorption culture.	HORSE, SHIGA.		Absorption culture.	HORSE, NEW HAVEN.		Absorption culture.	NORMAL RABBIT.		Absorption culture.		RABBIT 21/b.		Absorption culture.
	Before.	After.		Before.	After.		Before.	After.		Before.	After.	Before.	After.	Before.	After.	
{ Summer diarrhea, Y }	50	N. Haven	
	—	Manila	
	150	X	
	250	Sch/4	
{ Dyspeptic diarrhea, D/37 }	
	75	N. Haven	
	50	Manila	
	50	X	
{ Dysenterize, G/4 }	
	
	
	400	Sch/4	
{ Water, 21/b. }	20	N. Haven	Shiga
	
	
	50	—	21/b	
{ Water, 49/b. }	Shiga
	
	
	
{ Water, Sch/4. }	21/b
	
	
	

Absorption experiments with normal sera gave identical results with those obtained with the immune sera. Similar results were also obtained with the serum of an animal (rabbit) immunized with one of the water organisms when absorption tests were instituted, though in this instance absorption tests were made only with the Shiga bacillus and the organism employed in immunizing the animal.

The agglutination tests were made by means of the hanging-drop method and also by means of the "bioscopic" method of Neisser and Wechsberg (*Muenchener med. Wochenschr.*, 1900, no. 37). The results obtained with the first method compared very closely with those obtained later by the bioscopic method, and vice versa. Many of the tests were made by both methods, so that the results given in the tables represent frequently the mean of several tests. The results given in tables A and B represent usually as many as fifteen or twenty separate tests of the organism. For purposes of demonstration the bioscopic method is decidedly superior to the hanging-drop method, as it enables one to demonstrate at a glance the results of a series of tests.

The results obtained in these studies indicate that these water bacteria possess some receptors which are identical with those of *Bacillus dysenteriae*, as well as other receptors which are different in character. These facts are especially brought out in the effects of prolonged absorption of the agglutinins in the different sera, normal as well as immune, as well as in the marked increase in the agglutinins in the immune sera over those contained in the normal sera of different species of animals. The occurrence of these cross reactions between sera and different organisms has been known for some time. Durham (*Jour. of Experimental Medicine*, Vol. V., p. 353) presents a number of instances in which he encountered such cross reactions. He supposes that "a given agglutinin is not a single substance, but a complex one," the constituent haptophore of which he designates with capital letters, whilst the bacillary haptophores which are capable of giving rise to the formation of the agglutinins

when introduced into the animal body are represented by corresponding small letters.

Rodet and Lagriffont (Compt. rend. de la soc. de biol., 1902) have shown that through the immunization of an animal with *Bacillus coli communis* the serum acquires also agglutinating properties for *Bacillus typhosus*, and vice versa. They found this phenomenon to occur constantly, and they designate the two agglutinins in such sera as homologous and heterologous respectively. They are convinced that it is not brought about by a secondary infection with *Bacillus coli communis*. The homologous and heterologous agglutinins frequently bear a distinct quantitative relation to each other, such as 10:1, as when a typhoid immune serum agglutinates the typhoid bacillus in 1:10,000 dilutions and the colon bacillus in 1:1,000 dilutions.

Studies upon the biological characters of the water bacteria show that all except two belong in the colon group, and with three exceptions these belong in the class that ferment glucose, saccharose, and lactose. Three of the organisms of this group ferment only glucose and lactose. These bacteria appear to be similar to the organism which Lentz (Zeitschr. f. Hygiene, Bd. XLI., p. 559) has designated pseudo-dysentery bacillus. The two remaining organisms ferment none of the sugars, and from the fact that they produce an alkaline reaction in milk they may be regarded as corresponding with *Bacillus fecalis alkaligenes*. So far as could be determined, these two cultures correspond in every respect with *B. fecalis*. They appear to correspond with the organism which Kruse (Deutsche med. Wochenschr., 1901) designated pseudo-dysentery bacillus. If one may judge from these facts, the name pseudo-dysentery bacillus is a misnomer, and the mere fact that an organism agglutinates with the dysentery immune-serum is no indication that it bears any other relation to *Bacillus dysenteriae*. Some of the more important biologic and biochemic characters of the water bacteria are given in table C.

TABLE C.
Some of the Morphologic and Biochemic Characters of the Water Organisms.

CULTURES.	Motility.	Indol.	FERMENTATION OF SUGARS.				GROWTH IN LITMUS MILK.			
			Glucose.	Lactose.	Saccharose.	Mannite.	Reaction.	Coagulation.	Gas.	Reduction.
Water, 7/a	++	-	-	-	-	-	Alk.	No	No	No
" 9/b	++	+	+	+	+	+	Acid	Yes	Yes	Yes
" 21/b	+	+	+	+	+	+	"	"	"	"
" 43/a	++	+	+	+	+	+	"	"	"	No
" Sch/4	++	+	+	+	+	+	"	"	"	Yes
" Sch/5	++	+	+	+	+	+	"	"	"	No
" Sch/6	++	+	+	+	-	+	"	"	No	Yes
" Sch/b	+	+	+	+	+	+	"	"	"	"
" L/10	+	+	+	+	+	+	"	"	"	"
" L/11	+	+	+	+	+	+	"	"	Yes	No
" L/12	+++	-	-	-	-	-	Alk.	No	No	"
" 49/b	+	+	+	+	-	+	Acid	Yes	"	Yes
" 49/c	+	+	+	+	-	+	"	"	"	No

Briefly stated, the results of our studies seem to warrant the following conclusions:

1. The agglutination reaction with dysentery immune-serum cannot be relied upon in the differentiation of organisms of *Bacillus dysenteriae* group unless we know the limits of the agglutinating power of the serum employed for the particular organism against which the animal has been immunized.

2. The normal serum of the horse, rabbit, and dog contains agglutinins in relatively small amounts for a variety of organisms.

3. The immunization of an animal against a particular organism increases not only the agglutinins for that organism, but likewise induces an augmentation of the agglutinins of other organisms which are closely related in their receptor apparatus.

4. Absorption experiments show that the absorption of the agglutinins from a serum, by means of a particular organism, not only removes the agglutinins for the organism employed in the absorption experiment, but also some of the agglutinins for closely-related organisms.

5. In the absorption experiments upon the dysentery immune-sera, the water bacteria show a more marked influence upon the agglutinins of *Bacillus dysenteriae* (New Haven, for instance) in the serum of an animal immunized against this organism than do the organisms of the Philippine group or those isolated from cases of summer diarrhea.

6. Comparative studies of the biologic characters of the water bacteria show that none of them are true dysentery bacilli. They are similar to the organisms designated pseudo-dysentery bacilli by Lentz and Kruse, and are probably *Bacillus coli communis* and *Bacillus faecalis alkaligenes* respectively.

(In conclusion I desire to express my thanks to Dr. A. C. Abbott, Director of the Laboratory, for suggesting this investigation and for valuable advice during its progress.)

THE CONNECTION BETWEEN THE ALKALINITY OF CERTAIN BACTERIAL FILTRATES AND THEIR HEMOLYTIC POWER.¹

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The discovery by Ehrlich² of tetanolysin in the filtrates of broth cultures of the tetanus bacillus and the thorough study of this substance by Madsen³ have been followed by a number of investigations into the hemolytic properties of other bacterial filtrates. The broth cultures of several pathogenic microbes (*Staphylococcus*, *streptococcus*, typhoid bacillus, and others) and also those of certain saprophytic microbes (*B. megatherium* and some vibrios) have been found to yield filtrates that possess the power of hemolyzing various species of blood corpuscles. Several of these bacterial hemolysins, like the hemolysins that are normally present or are artificially produced in blood sera, are inactivated by exposure for thirty minutes at a temperature of 55°–56° C. An exception to this occurs in the case of the hemolysin elaborated by *streptococcus*, which, according to Besredka,⁴ is not inactivated at 55° in less than ten hours, although at 70° it is destroyed in two hours. The hemolysins produced by *B. pyocyaneus* and *B. typhosus* are still more resistant and, according to several writers, cannot be destroyed by temperatures of 100° C. and upward.

This singular property of heat-resistance shown by certain of the bacterial hemolysins indicates that the hemolysing substance in these cases is of a very different character from the hemolysins of blood sera. The hemolysin is not necessarily a body of complex constitution. The well-known laking effect produced by alkalis and the fact that many bacterial filtrates possess a strongly alkaline reaction led me to examine the relation existing between the alkalinity of the

¹ Received for publication May 21, 1903.

² Berl. klin. Wochenschr., 1898, No. 12, p. 273.

³ Zeitschr. f. Hyg., 1899, xxxii, p. 214.

⁴ Annales de l'Institut Pasteur, 1901, xv, p. 880.

bacterial filtrate and the power of the filtrate to produce hemolysis.

The alkaline reaction of certain cultures of *B. pyocyaneus* first suggested the quantitative examination of the effect of alkali upon blood of various kinds.¹ That some relation exists between the alkalinity and the hemolytic power of filtrates of this organism was noticed by Lubenau² who observed that a filtrate of *B. pyocyaneus*, when brought to the litmus neutral point, exerted a more feeble hemolytic action than the unneutralized filtrate. Very recently Kayser,³ in a paper on "colilysin," remarks on the connection between alkalinity and the ability to produce hemolysis, and supplements Lubenau's observation by noting that neutralization materially lessens the hemolytic power of the coli filtrates. In one instance Kayser records the fact (*loc. cit.*, Table VII., p. 129) that one cubic centimeter of a hemolyzing filtrate, after being rendered neutral, produced only as much hemolysis as 0.025 of a cubic centimeter of the same filtrate before neutralization! Neither Kayser nor Lubenau, however, appear to have conducted control experiments with other fluids of the same degree of alkalinity as the filtrates.

Methods.—Those investigators who have worked with bacterial hemolysins have employed quite different methods. The blood that has been used has been suspended in 0.85 per cent NaCl solution (most investigators) and in 0.6 per cent NaCl solution (Bullock and Hunter); the period of incubation at 37° has varied greatly (Kayser, two hours; Bullock and Hunter, eighteen to twenty hours; Weingeroff, "until laking occurred."); the quantity of blood on which the filtrates were allowed to act has also been very different; so that in few cases can any precise and satisfactory comparison be made between the results of different experimenters.

The writer has used in his work the methods in vogue in many of the more recent experiments with hemolytic blood

¹ Cf. Transactions Chicago Pathol. Soc., Dec. 8, 1902, v, p. 175.

² Centralbl. f. Bakt., 1901, xxx, p. 402.

³ Zeitschr. f. Hyg., 1903, xlii, p. 118.

sera: for the test-object, freshly drawn defibrinated blood has been used in a five per cent suspension in 0.85 per cent NaCl solution; one cubic centimeter of the suspension in small test tubes of uniform bore (.8 cm.) has been taken as the standard quantity for testing hemolytic power. To the suspension has been added the hemolyzing fluid, and the mixture then incubated at 37° C.; it has been next placed in the ice-box at 10°–12° C. for twenty to twenty-four hours, except when otherwise stated. Control tubes were always incubated with the others, and any series showing even a trace of hemolysis in the controls has been discarded.

Unwashed corpuscles have been used in the experiments described in this paper. The sensitiveness of dog and rabbit corpuscles to alkali and to "pyocyanolysin" is slightly increased by washing, but the difference is comparatively trivial, and was found not to influence the general character of the results.

NaCl Solution. — Tests have been made to determine the amount of hemolysis produced by simple NaCl solution (0.85 per cent), to which measured amounts of normal alkali (NaOH) have been added. The alkalinity of such solutions — and their hemolytic power — diminishes very rapidly on exposure to the air, and the solution must be freshly prepared with carefully titrated normal NaOH solution if comparable results are to be obtained. Different kinds of blood corpuscles show different degrees of susceptibility to the action of alkali, as to other hemolytic agencies. The following table illustrates some of the differences observed:

TABLE I.

1 cc. of 0.85 per cent	Dog.	Hog.	Cow.	Sheep.	Rabbit.
NaCl solution.....	o	o	o	o	o
" " containing, of normal NaOH, 1 p.c.	Complete	Almost complete	Almost complete	Almost complete	Complete
" " " .5 "	"	Almost complete	Fair	Strong	"
" " " .2 "	Very strong	o	Tr.	o	Very strong
" " " .1 "	Very strong	o	o	o	Tr.
" " " .07 "	Strong	o	o	o	—
" " " .05 "	Tr.	—	—	—	o
" " " .04 "	Tr.	—	—	—	—
" " " .03 "	Tr.	—	—	—	—
" " " .02 "	o	—	—	—	—
" " " .01 "	o	—	—	—	—

When the mixtures of blood and alkaline NaCl solution are incubated at 37° C. for longer than one hour before being placed on ice, the hemolytic action of the alkali is more pronounced. The "latent period" observed by Madsen (op. cit., p. 220) in the action of tetanolysin occurs also in the case of the simple alkaline NaCl solution.

TABLE II.

1 cc. of 0.85 per cent NaCl solution, containing .2 per cent normal NaOH.	Rabbit.	Guinea-pig.
Incubated at 37°, 1 hr.....	Tr.	Tr.
" " " 2½ hrs.	Tr.	Tr.
" " " 22 hrs.	Complete	Very Strong
Control incubated at 37°, 22 hrs.....	o	o

Nutrient Broth. — When alkali is added to broth of a neutral reaction (indicator, phenolphthalein), the hemolytic power of the resulting mixture is, as might be expected, not so great as when the same amount of alkali is added to simple sodium chloride solution. One cubic centimeter of a .5 per cent alkaline NaCl solution, for example, completely hemolyzes dog's blood (one cubic centimeter of a five per cent suspension) in one hour at 37° C., while the same amount of broth to which .5 per cent of normal alkali has been added usually fails to produce complete hemolysis and in some instances yields only a trace. The reason for the difference appears to be that some of the hydroxyl ions, introduced into the broth in adding the normal alkali solutions, are anchored by some substance (or substances) in the broth, probably the same as that which falls as a well-known proteid precipitate (alkali albumen) when the alkalinity reaches a certain point. Different lots of broth behave somewhat differently even when the neutral point is determined with precision and when exactly equivalent amounts of alkali are added. This variation probably depends upon fluctuations in the amount of the substances that are able to bind the hydroxyl radical. If the broth be made quite strongly alkaline, for instance by adding twenty cubic centimeters of normal NaOH per liter, so that a precipitate forms, and the precipitate is removed by filtration, the broth may be reduced to one per cent of alkalinity (equivalent to ten cubic centimeters of normal alkali per liter), and its hemolytic behavior will then exhibit a close correspondence with the simple NaCl solution of the same degree of alkalinity. Broth from which the precipitate has been removed in this way has been used in the experiments presently to be described. Like the NaCl solution, the broth rapidly loses its alkalinity on standing.

B. pyocyaneus. — Kraus and Clairmont¹ were the first to test the laking power of the products of *B. pyocyaneus*, and observed that a one-day old living culture had no hemolytic effect upon rabbit blood. Bullock and Hunter,² however,

¹ Wiener klin. Wochenschr., 1900, p. 49.

² Centralbl. f. Bakt., 1900, xxviii, p. 865.

found that while young cultures of *B. pyocyaneus* contained no hemolysin, older cultures and the filtrates obtained from these cultures exerted a hemolyzing power upon the blood-corpuscles of the rabbit, sheep, dog, ox, and other animals. Weingeroff,¹ Lubenau,² and Breymann³ have also studied the hemolytic effect of cultures and filtrates of *B. pyocyaneus*, and although their methods have differed widely, the results and conclusions of these authors have been substantially the same, and "pyocyanolysin" has been usually included in the list of bacterial hemolysins.

In the series of experiments reported in this paper both cultures and filtrates of *B. pyocyaneus* have been tested for hemolytic power. In agreement with Weingeroff and Breymann, it has been found that the hemolytic quality is not destroyed by heat (125° C. in the autoclav for one hour). Bullock and Hunter state that the filtrates part with their hemolytic power to some extent on the application of heat, while the cultures do not, but I have not noted any difference between culture and filtrate in this respect, as in fact Breymann also failed to do. The use of these high temperatures eliminates the ordinary proteolytic enzymes which might otherwise complicate the results.

In all cases an intimate connection has been found to exist between the degree of alkalinity of the filtrate and its hemolytic power. The initial reaction of the nutrient broth affects to some extent the amount of alkali developed by the culture. The following table illustrates the increase in alkalinity of two flasks of sugar-free broth,⁴ which were similar before inoculation, except in regard to their reaction; the flasks were inoculated with the same culture of *B. pyocyaneus* at the same time and incubated at 37° C.

¹ Centralbl. f. Bakt., 1901, xxix, p. 777.

² Centralbl. f. Bakt., 1901, xxx, p. 402.

³ Centralbl. f. Bakt., 1902, xxxi, p. 481.

⁴ It is well known that the presence of sugar in the culture medium influences the reaction of the medium in those cases where the bacillus is able to ferment the sugar. To avoid this complication I have in all instances removed the muscle sugar by Smith's method. The presence of sugar might conceivably prevent the development of hemolysin, provided the alkalinity and the hemolysin were one and the same.

TABLE III.

Initial reaction.	A 1.0 acid.	B neutral.
1 day3 acid	.2 alkaline
2 days1 "	.2 "
3 "1 alkaline	3. "
4 "5 "	.9 "
5 "5 "	.9 "
7 "	1.0 "	1.2 "
9 "9 "	2.0 "
12 "	1.6 "	2.3 "
16 "	1.6 "	2.3 "

It is important that the titration be carried out in a uniform manner if comparable results are to be obtained. In the work recorded in this paper the reactions have in all cases been determined as follows: Five cubic centimeters of the fluid to be tested have been placed in a porcelain evaporating dish with forty-five cubic centimeters of distilled water previously boiled to expel the carbonic acid. After boiling for about two minutes the titration is made with $\frac{N}{20}$ NaOH or $\frac{N}{20}$ HCl, using one cubic centimeter of phenolphthalein solution as the indicator. It is necessary to titrate while boiling, or when the solution is exactly at the boiling point, otherwise an exact end point is not obtained. The filtrates of *B. pyocyaneus* are often difficult to titrate with complete precision, owing to their high color, but with practice closely comparable results can be secured.

Cultures that have attained the degree of alkalinity shown by the four to five day old cultures in Table III., namely about .5 per cent, are able to hemolyze blood corpuscles of various kinds. It does not appear, however, from any of the experiments made that the hemolyzing power of these filtrates is any greater than might be expected to result from the simple alkalinity of the medium. In one instance, which

may serve as typical of a large series, comparison of the hemolytic action of a one per cent alkaline NaCl filtrate of *B. pyocyaneus* with ordinary sterile nutrient broth of the same degree of alkalinity and with one per cent alkaline NaCl solution (.85 per cent) gave the following results:

TABLE IV.

	1 cc. of a 5 per cent suspension of Dog's Blood.			1 cc. of a 5 per cent suspension of Rabbit's Blood.		
	Pyocyan- eus fil- trate. 1 per cent alkaline.	Broth. 1 per cent alkaline.	NaCl solution. 1 per cent alkaline.	Pyocyan- eus fil- trate. 1 per cent alkaline.	Broth. 1 per cent alkaline.	NaCl solution. 1 per cent alkaline.
1 cc.	Complete	Complete	Complete	Complete	Complete	Complete
.5 cc.	"	"	"	Strong	"	"
.1 cc.	"	"	"	o	Trace	Trace
.05 cc.	Distinct	Distinct	Distinct	o	o	o

1 hour at 37°; 22 hours at 12°.

Seven different strains of *B. pyocyaneus* have been employed in tests of this character and all have given substantially the same results. Six of these strains had been under cultivation for some time and one of them had been increased in virulence by passage through the bodies of guinea-pigs; the seventh strain was freshly isolated from a case of acute gangrenous appendicitis and was quite virulent, one-half cubic centimeter of a broth culture proving fatal to a guinea-pig in twenty-two hours. The more virulent cultures did not manifest appreciably greater hemolytic power than the others. In all cases diminution of the alkalinity correspondingly diminished the hemolytic power of the filtrates; filtrates that were rendered neutral to phenolphthalein were no longer hemolytic.¹ Filtrates of *B. pyocyaneus* and of certain other

¹ This is true for the ordinary period of incubation (one hour), but on longer exposure (twenty hours or more) both neutral pyocyanus filtrates and neutral broth produce some hemolysis with dog's blood, while a control tube of one per cent acid broth gives no hemolysis. As is well known, however, broth that is neutral to phenolphthalein is alkaline to litmus and hence must contain some hydroxyl ions.

bacteria, which for one reason or another react slightly acid to phenolphthalein, will produce hemolysis if they are brought to the same degree of alkalinity as the naturally hemolytic filtrates. It may be noted that the conditions which Loew and Kozai¹ found favorable to the development of the hemolytic properties of *B. pyocyaneus*, namely, exposure of a large surface of the culture to the air, are precisely the conditions that lead to the production of a strongly alkaline reaction.

The statements found in the literature concerning "pyocyanolysin" do not forbid the assumption that the hemolytic effects that have been observed by other investigators have also been due to the alkalinity of the respective filtrates. The relatively long contact necessary for a manifestation of the hemolytic power (eighteen to twenty hours at 37°, Bullock and Hunter), the particular susceptibility of the dog's corpuscles² (Breyman), and the resistance of the hemolysin to high temperature are facts that entirely accord with the view that the hemolytic properties of *B. pyocyaneus* filtrates are due at least in large part simply to the alkalinity of the fluid.

In none of the papers on pyocyanolysin that have come to my notice is any statement made regarding the keeping qualities of this substance. Kayser, however, in his recent paper on colilysin, records the observation that the latter hemolysin, which is also heat-resistant, loses its strength on standing, even if a preserving fluid of carbol and glycerine be added, but maintains its strength if well protected from the air in a rubber-stoppered flask. I have found that the filtrates of *B. pyocyaneus* lose their strength in a similar fashion

¹ Bulletin of the College of Agriculture, Tokio, 1902, 4, p. 323.

² Other bacterial hemolysins of the thermo-stabile class appear from the statements of authors to hemolyze canine corpuscles more readily than other blood corpuscles. "The most suitable blood" for testing the presence of typholysin is said by Castellani (*Lancet*, i, 1902, p. 440) to be that of the dog, and according to this writer the same thing applies to the hemolysin produced by the dysentery bacillus. Colilysin (Kayser) also acts most strongly upon dog corpuscles. A simple alkaline NaCl solution, as I have already pointed out, has the same predilection. Over against this may be put the action of the thermo-labile hemolysins such as staphylolysin and tetanolyisin, which do not display any marked affinity for the dog's corpuscles, and which hemolyze in different degrees the corpuscles of different animals. Besredka has shown that different streptococcolysins are formed according to the medium (different blood-sera) upon which the streptococcus is grown.

on standing, and have interpreted this as due to loss of alkalinity caused by the action of the atmospheric carbon dioxide. Precisely the same change has been noted in the alkaline broth and the NaCl solution, and is a generally familiar chemical phenomenon. Kayser, as a matter of fact, observed a decrease in alkalinity in the filtrates of *B. coli* on standing, but apparently failed to trace any causal connection between this and the coincident loss of hemolytic power.

The action of CO_2 on pyocyanolysin is shown by the following experiment :

TABLE V. — DOG'S BLOOD.

	Untreated pyocyanous filtrate.	Pyocyanous filtrate through which a stream of CO_2 had been passed for 30 minutes.	Pyocyanous filtrate through which a stream of O_2 had been passed for 30 minutes.
1 cc. . .	Complete	o	Complete
.5 cc. . .	Complete	o	Complete
.3 cc. . .	Almost complete	o	Complete
.1 cc. . .	Decided	o	Very strong
.05 cc. . .	Tr.	o	Tr.
Control . .	o		

The passage of CO_2 through the filtrate destroys the alkalinity as well as the hemolytic power, while the passage of O_2 leaves the alkalinity substantially unaffected. On heating the CO_2 filtrate the CO_2 is expelled and the filtrate regains at once its alkalinity and its hemolytic power. Alkaline NaCl solution behaves in precisely similar fashion.

In many particulars, therefore, the conditions that influence the alkalinity of the fluids containing the thermo-stabile bacterial hemolysins affect also the hemolytic properties of these fluids. The amount of hemolysin exists, rises and falls in a heated filtrate in such exact correspondence with the

alkalinity that it is impossible to avoid the conviction that the "hemolysin" and the active hydroxyl ion are one and the same.¹ If it be true that any salts of weak acids or strong bases that can dissociate to yield hydroxyl ions can produce hemolysis, then very definite evidence should be adduced to justify the hypothesis that a more complex substance or specific hemolysin exists in markedly alkaline bacterial filtrates.

The fact that several authors assert that "anti-hemolysins" can be produced by injection of certain of the thermo-stabile bacterial hemolysins might seem to indicate that in these cases the action of the alkali is super-imposed upon, or perhaps reinforces the action of a "true" hemolysin in the way that proteolytic enzymes are favored by the presence of alkali, but further evidence is needed upon this point. I have not yet been able to obtain an "anti-pyocyanolysin" by injection of heated hemolytic pyocyaneus filtrate, although several animals have been treated for this purpose.² It is of course possible that strains of *B. pyocyaneus* exist which are able to produce a thermo-stabile hemolysin distinct from simple alkali, but this assumption needs definite corroboration.

¹ This may be contrasted with the statement made by Neisser and Wechsberg (*Zeitschr. f. Hyg.*, 1901, 36, p. 299) concerning the effect of altering the reaction of the filtrate containing staphylolysin: "Bemerkt werde noch, dass der Alkalitätsgehalt der Bouillon, so bedeutend er zwar für die *Production* des Hämolymins ist, für die *Wirkung* des Hämolymins auf die rothen Blutkörperchen von verhältnissmässig nebensächlicher Bedeutung ist. Man kann nämlich einem einmal producirten Hämolymin in relativ weiten Grenzen Alkali oder Säure (oder Kochsalz) zusetzen, ohne die Lösung der Erythrocyten wesentlich zu beeinflussen."

² For example, one rabbit, after receiving seventy-four cubic centimeters of hemolytic pyocyaneus filtrate intraperitoneally, and another after receiving twenty-eight cubic centimeters subcutaneously at appropriate intervals have yielded no specific "anti-pyocyanolysin."

A STUDY OF THE PROTEOLYTIC ENZYMES AND OF THE
SO-CALLED HEMOLYSINS OF SOME OF THE COMMON
SAPROPHYTIC BACTERIA.*

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One of the results of Ehrlich's elaborate studies of infection and immunity is the conception of a fundamental similarity between the mechanisms of intoxication and assimilation.¹ The destiny of both toxins and assimilable matters in the living body he conceives to be determined by the presence or absence in the body-cells of special atom groups endowed with the power to unite with appropriate atom groups in the toxin or the assimilable molecule. The presence or absence in the body of such special atom groups, "receptors," as they are designated, because of their specific affinity for the toxin, decides whether a given toxin shall or shall not be toxic for the particular living organism under consideration, *i.e.*, decides the receptivity or susceptibility of the tissues for the poison. In a similar manner the capability of other extraneous substances for good, instead of harm, that is, for the nourishment instead of the destruction of the tissues, is likewise conceived as dependent upon the possession by certain cells or groups of cells, of "receptors" having a similar power to combine with and utilize the assimilable or nutritious matters. In the case of toxins the union with the tissue is destructive, in that of the assimilable matter it is constructive. Both toxins and nourishing substances are believed to combine with their respective receptors through the agency of the so-called "haptophore" groups; *i.e.*, that part of their molecules whose function it is to bind

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them to the respective cell for which they are destined to enter into intimate chemical union.

Viewed from this standpoint it is obvious that the ingenious deductions of Ehrlich are of much broader biological significance than when restricted to the phenomena of infection and immunity as ordinarily understood.

The wealth of experimental data that has resulted from the enthusiastic pursuit of studies in this field contains sufficient to warrant the belief that this conception is far from fantastic. In many particulars demonstrations of a most surprising character have been made. The induction of systemic reactions, characterized by the appearance of specific antidotes in the blood, through the injection of animals with such matters as milk, alien sera, definite ferments, and various types of cells hitherto regarded as scarcely more than inert, and never as poisons, cannot be said to have been expected; and the analyses of the mechanism through which these reactions occur have shown them to conform, in the main, to the same laws that are now believed to govern the action of many well-known harmful intoxicants.

It was this interesting conception that led us to the study of certain ordinary non-pathogenic bacteria with the hope of discovering the relation of themselves or their products to the living tissues, and of deciding by what mechanism their products of growth, often active in special ways in the test tube, are caused to disappear from the living animal without having been at any time after their introduction the cause of noticeable disturbance of physiological function or structure.

The organisms included in this study are of the actively liquefying type, *i.e.*, the elaboration of proteolytic enzymes is one of their conspicuous physiological properties. They have also, as we discovered later, the power of causing more or less hemolysis of mammalian erythrocytes. It was to these characteristics that our attention was particularly directed.

The course taken by our experiments was determined by the following main questions:

Is it possible, through the use of the products of non-pathogenic bacteria, to induce in the living animal systemic reactions that are characterized by the elaboration of specific anti-bodies (anti-enzymes) in the circulating blood?

Can such reactions be utilized in deciding the destiny of these products in the body?

Will it be possible in this manner to demonstrate the relation of the bacterial products to one another and to analogous components of the normal animal body?

Is the mechanism of the hemolysis that results from the action of living saprophytic bacteria or the products of their growth the same as that of an immune serum upon the erythrocytes from which the animal supplying it is immunized? Is it the result of the digestive action of proteolytic ferments upon the stroma of the erythrocytes, thereby liberating the hemoglobin? Or is it a physical phenomenon based upon alterations in tonicity or chemical composition of the fluid in which the corpuscles are located?

The bacteria chosen for the investigation were *B. fluorescens* and *B. pyocyaneus*, not only because of their apparent botanical relationship, but because of the hemolytic activity already demonstrated for the products of growth of *B. pyocyaneus*; *B. subtilis*, because of its apparent relationship to *B. megatherium*, which Todd³ has shown to possess certain points of decided interest; and *B. prodigiosus* and *B. proteus* (*vulgaris*), because of the activity of the proteolytic enzymes produced by them under a great variety of conditions.

In certain particulars our questions have been satisfactorily answered, in others not; but on the whole we believe our study has furnished results that are not without some degree of interest and importance, and we therefore embody them in this communication.

In approaching our problem it was decided to employ both for immunization and for hemolytic tests the filtered products of growth of the several bacterial species named, after they had developed under a variety of nutritive conditions for varying periods of time.

The filtration was uniformly through the Berkefeld filter under negative pressure.

The object upon which the proteolytic activity was tested was five cubic centimeters of a carbolized gelatin made as follows :

Gelatin — 8 cubic centimeters.

Phenol — 0.25 cubic centimeters.

Water — 100 cubic centimeters

The reaction of which corresponded to eight points on the alkaline side as represented in Fuller's scale.

The phenol was added for the purpose of eliminating error through the growth of bacteria ; control tests having shown that in this strength it had no influence upon enzyme action.

The amount of filtrate employed for determining proteolytic activity varied, but was most often from 0.25 cubic centimeters to 0.5 cubic centimeters, though in the majority of our active filtrates complete liquefaction resulted in from eighteen to twenty-four hours with amounts as low as 0.01 cubic centimeter.

In some cases the filtrate was deposited upon the horizontal surface of the gelatin and its digestive activity determined by the rate at which the level of the solid gelatin was lowered ; in others, and perhaps most frequently, the filtrate was thoroughly mixed with the liquefied gelatin at about 35° C., and in the majority of cases was then kept for varying periods at 38° C., after which it was placed in ice water and the extent of digestion determined by the more or less complete failure of the mass to solidify. The activity of the filtrate was determined by the time required for complete liquefaction of the mass. Without entering into needless details it suffices to say that by this plan our preliminary studies afforded the following information :

The gelatin-liquefying activity of the filtrate, *i.e.*, its enzyme content, presumably, is in large part dependent upon the character of the nutrient medium in which the bacteria were cultivated. The minimum evidence of digestion was given by filtrates from the non-proteid culture media. The enzyme content of bouillon cultures was with certain species

at times almost nothing after a reasonable period of growth, while at other times and constantly with other species it was more or less marked. The enzyme content of completely liquefied gelatin cultures was always marked, and was in general more marked for all species than was the case with any of the other culture media used by us. This is not due to the factor of time, for bouillon cultures of the same age as the completely liquefied gelatin cultures were uniformly less active in causing liquefaction of the carbolized gelatin than was the filtrate from the gelatin culture.

When the organisms employed are cultivated in nutrient gelatin there is, as is known, some degree of liquefaction to be observed almost as soon as growth is certainly evident. If, on the other hand, they be cultivated in media free from gelatin, growth may be active, but enzyme production be so inactive that twenty-four to thirty-six hours may be required for the elaboration of sufficient of the ferment to be certainly demonstrated by the method of testing used by us.

One of two interpretations may be offered for this: Either the enzyme in the gelatin free fluid is so diluted that its presence cannot be detected until time has elapsed for the elaboration of moderately large amounts, or — and we incline to this view — the bacteria growing in gelatin are stimulated to enzyme production through the specific action of the gelatin upon those atom groups of the bacterial protoplasm that are concerned in the elaboration of the ferment. We are not, as we see it, without an analogue for this. In his beautiful research upon gastric secretion, Pawlow demonstrates that, next to psychic influences, *i.e.*, appetite, the stimuli of greatest potency in calling forth secretion are, in the main, those food stuffs on which the gastric juice exhibits its most characteristic properties. Interpreted in the light of Ehrlich's doctrine, those substances find, presumably in the gastric cells, receptive atom-complexes for which they possess specific affinity and with which they unite — the union serving as a stimulus to the over-production by the cell of that particular receptive atom group. The excess of such groups,

resulting in this way, escape from the cell and constitute the proximate principle of the juice.* We conceive the action of the gelatin in inducing active elaboration of enzyme by the bacteria to be explainable in a similar manner.

As is the case for the majority of proteolytic enzymes, be their origin what it may, we find our filtrates to be uniformly more active when they are of alkaline than of neutral or acid reaction. When acidified they are, as a rule, inactive. In a similar manner their production by the growing organism is always more marked in alkaline than in either neutral or acid media, even though the latter is not sufficient to depress growth to any marked extent. The differences in digestive activity of the several filtrates, from the standpoint of reaction, are too slight for this to serve as a means of distinguishing one from another.

In their relations to heat, some degree of difference between the several filtrates is detected, and in certain cases there are very great differences between our results and those recorded by other investigators.

The most noteworthy investigations upon the properties of proteolytic ferments of bacterial origin are those of Fermi.⁴ In the main, our results agree with those contained in Fermi's papers, but in one particular they differ widely. In the work mentioned, and generally since its publication, it is stated that the proteolytic ferments, both those of animal and those of vegetable origin, are destroyed when in the moist state by a brief exposure to about 70° C. In some cases even a lower temperature is said to destroy them, in others a somewhat higher may be withstood. We have found that the filtrates from the organisms used by us with two exceptions, *viz.*, *B. pyocyaneus* and *B. subtilis*, can be heated to 100° C. for, in some cases, as long as fifteen to thirty minutes, without losing the power to liquefy carbolized gelatin. The rate of their action is diminished, it is true, but this has been the only difference observable between the heated and the unheated enzymes.

* In yielding to the temptation to interpret this observation in terms of Ehrlich's doctrine, we are aware that this is not Pawlow's view of the phenomenon. He conceives it to be at bottom a nervous function.

The tests were made in several ways in order that they might be sufficiently controlled by heating the filtrates in the water bath at boiling temperature in test tubes; by inclosing the filtrates in sealed tubes and keeping them immersed in boiling water; and by heating in tubes or flasks at the temperature of streaming steam in the sterilizer. Aside from inconsiderable variations, the results were uniform, *viz.*, as stated, these organisms elaborate proteolytic enzymes that, even in the moist state, are capable of resisting the destructive action of 100° C. for such long periods as fifteen to thirty minutes.

Through the investigations of Roden,⁵ Hahn,⁶ Morgenroth,⁷ Landsteiner,⁸ Achalme,⁹ Fermi,¹⁰ von Dungern,¹¹ and others, a number of facts of direct bearing upon our problem have been established; notably, that there are normally present in the circulating blood of many mammals bodies that have the power to inhibit the activity of some of the important enzymes, not only those elaborated by the animal organism, but those found in the vegetables as well, and that in some cases the repeated artificial introduction of such enzymes into the living animal calls forth systemic reactions that make themselves manifest through the development of specific anti-bodies, demonstrable in the blood. It is, however, seldom possible to induce in the treated animals very high degrees of immunity, and never to the same extent as that often seen to follow the use of active toxins, probably for the reasons that the enzymes used for injection, or enzymes closely allied to them, are normal constituents of the living animal, and are, therefore, incapable of stimulating the tissues, by nature more or less tolerant to them, to the same degree as is possible with fully alien substances to which the cells are unaccustomed.

In our experiments more or less similar conditions were encountered. The most characteristic feature of the organisms used by us is their power to liquefy gelatin, that is, to elaborate proteolytic enzymes. In the test tube both the growing organisms and the products of their growth bring about this change in gelatin with unusual rapidity, and yet

both the living organisms and the filtered products of their growth, particularly the latter, may, with an occasional exception, be injected into rabbits, often in very large amounts, without causing noticeable disturbance. If the injection be frequently repeated, one can after a little while employ really enormous doses without serious consequences. On reaching this point with the animals upon which we were experimenting they were bled, and the reactions between their blood serum and the filtered bacterial products determined. The results were interesting. In all cases the sera were to a greater or less extent restraining for the liquefying enzymes, but in no case very markedly so. The serum from an animal that had received a number of injections of a special filtrate was uniformly more active in restraining the proteolytic activity of that particular filtrate than of the others in the group, but it was not always much more active. This led us to suspect that a certain increment of the restraint exhibited by the "immune" serum was, perhaps, common to normal serum, as noted by other investigators working with other enzymes, and on testing the action of normal serum upon the several filtrates under consideration we found this to be the case; the proteolytic activity of all disappeared when normal rabbit serum was mixed with them, though as a rule a larger amount of the normal than of the immune serum was needed to produce this result, especially when the immune sera were mixed with their corresponding filtrates.

Heating both the normal and the immune sera to 56° C. for thirty minutes has, to our surprise, little or no appreciable effect upon their restraining action. It is probable that a more systematic study of both the normal and the immune sera from the standpoint of their thermal reactions would have furnished more instructive results.

Our results convince us, however, that it is possible to distinguish through the use of such "immune" sera, poor though they may be in specific anti-body, the several filtrates from one another as well as to demonstrate a difference between them and that proteolytic enzyme of animal origin with which they have many points of resemblance — namely, trypsin.

While we found the activity of pancreatin solution (0.3 to 1 per cent of Merck's pancreatin in 0.5 per cent solution of Na_2CO_3) to be checked by both normal serum and that of rabbits treated with the bacterial enzymes, we have regularly noted the latter to be no more actively restraining than the former. It is always possible, moreover, to distinguish between the pancreatin solution and the solutions of bacterial proteolytic enzymes, used for immunizing the animals, by the greater restraining power of the "immune" serum upon its specifically related enzyme than upon either the enzyme of animal origin or those of bacterial origin constituting the remaining members of the group considered by us.

The reactions are, perhaps, best illustrated by the following protocol:

Showing the influence of various enzyme solutions alone and with normal and immune rabbit sera upon five cubic centimeters of carbolized gelatin of slightly alkaline reaction at room temperature.

Pancreatin solution = 1 gram pancreatin in 100 cc. of 0.5 per cent Na_2CO_3 solution.

0.5 cc. pancreatin sol. = complete digestion* in 2 hours.

0.5 cc. pancreatin sol. + 0.5 cc. normal serum = digestion completely arrested.†

0.5 cc. pancreatin sol. + 0.5 cc. fluorescent immune serum = digestion completely arrested.

0.5 cc. pancreatin sol. + 0.5 proteus immune serum = digestion completely arrested.

0.5 cc. pancreatin sol. + 0.5 pyocyaneus immune serum = digestion completely arrested.

* By "complete digestion" is meant that in the time given the influence of the filtrate upon the gelatin was such that the gelatin was completely liquefied and could not be again solidified at the temperature of melting ice.

† By "digestion completely arrested" is meant that there was no evidence of liquefaction of the gelatin at any time within five days after making the test. In most cases this was true for longer periods, but five days was taken as a fair standard.

Filtrate from gelatin culture of *Bacillus fluorescens* :

- 0.25 cc. filtrate = complete digestion in 2 hours.
- 0.25 cc. filtrate + 0.2 cc. *fluorescent immune serum* = *digestion completely arrested.*
- 0.25 cc. filtrate + 0.5 cc. normal serum = digestion complete in between 18 and 48 hours.
- 0.25 cc. filtrate + 0.5 cc. pyocyaneus immune serum = complete digestion in 18 to 48 hours.
- 0.25 cc. filtrate + 0.5 cc. proteus immune serum = complete digestion in 48 to 72 hours.

Filtrate from gelatin culture of *B. proteus (vulgaris)* :

- 0.25 cc. filtrate = complete digestion in 2 hours.
- 0.25 cc. filtrate + 0.25 cc. *proteus immune serum* = *digestion completely arrested.*
- 0.25 cc. filtrate + 0.5 cc. normal serum = digestion complete in between 72 and 120 hours.
- 0.25 cc. filtrate + 0.5 cc. pyocyaneus immune serum = digestion complete in 96 to 120 hours.
- 0.25 cc. filtrate + 0.5 cc. *fluorescens immune serum* = digestion complete in 72 to 120 hours.

Filtrate from gelatin culture of *B. prodigiosus* :

- 0.25 cc. filtrate = complete digestion in 2 hours.
- 0.25 cc. filtrate + 0.5 cc. normal serum = digestion complete in between 18 and 48 hours.
- 0.25 cc. filtrate + 0.5 cc. *fluorescens immune serum* = digestion complete in between 4 and 18 hours.
- 0.25 cc. filtrate + 0.5 cc. pyocyaneus immune serum = digestion complete in between 18 and 48 hours.
- 0.25 cc. filtrate + 0.5 cc. proteus immune serum = digestion complete in between 18 and 48 hours.

Filtrate from gelatin culture of *B. pyocyaneus* :

- 0.25 cc. filtrate = complete digestion in 2 hours.
- 0.25 cc. filtrate + 0.1 cc. *pyocyaneus immune serum* = *digestion completely arrested.*

0.25 cc. filtrate + 0.5 cc. normal serum = digestion complete in between 8 and 18 hours.

0.25 cc. filtrate + 0.5 cc. fluorescens immune serum = digestion complete between 8 and 18 hours.

0.25 cc. filtrate + 0.5 cc. proteus immune serum = digestion complete in less than 18 hours.

From the foregoing we see that in the amounts used both pancreatin solution and the filtrates of the liquefied gelatin cultures caused complete liquefaction (digestion) of the carbolyzed gelatin in two hours; that the addition of both normal and immune sera to the solutions materially postponed the date of complete digestion, but that in no case is the postponement as great as where an immune serum acts upon its specifically related filtrate.

The histories of the immune sera used in the foregoing tests were as follows:

Fluorescens immune serum. — This serum came from two rabbits, Nos. 6 and 7.

Rabbit No. 6 received twelve intraperitoneal injections over a period of twenty-eight days. The total amount of filtrate injected was 157 cc. The filtrate was from a culture one hundred and twenty hours old. The minimum dose was 10 cc., the maximum 15 cc.

Rabbit No. 7 received eight injections, likewise intraperitoneal, in thirteen days. The total amount of filtrate used was 115 cc. of a completely liquefied gelatin culture. The minimum dose was 10 cc., and the maximum 20 cc. of the filtrate.

Proteus immune serum — The rabbit supplying this serum received twenty-one intraperitoneal injections running over a period of forty-one days. In all it received 219 cc. of sterile filtrate from completely liquefied gelatin cultures eight to twelve days old. The minimum dose was 6 cc., and the maximum 15 cc.

Pyocyaneus immune serum. — The rabbit from which this serum was obtained received twenty-two intraperitoneal injections over a period of forty-three days. It received in all 307 cc. of sterile filtrates from gelatin cultures of from six to fifteen days old. The minimum dose was 12 cc., the maximum 20 cc. of the filtrate.

An interesting outcome of the study of bacteria and their products by the newer methods of investigation is the demonstration that many of them elaborate in the course of

their growth substances having a destructive action upon the blood, both in vitro and in vivo. These lysins, or hemolysins, as the hemolyzing agents are called, suggest by their action, in some cases, the proteolytic enzymes; in others their molecule appears to be a composite of haptophore and toxophore groups, the former being more or less stable in their relation to heat, and having the power of binding the lysin to the susceptible cell, the latter being unstable to heat, but having the power of destroying the cell to which the haptophore links it; while in still other instances the destruction of blood, particularly in vitro, seems to result from detrimental physical and chemical conditions brought about by the growing bacteria, or presented by the filtered products of their growth.

The studies that have been made, for instance, upon *B. tetani*, *Staphylococcus pyogenes aureus*, *Streptococcus pyogenes*, *Bacillus coli communis*, *B. typhosus*, *Bacillus pyocyaneus*, and *Bacillus megatherium* reveal the interesting fact that the products of all are more or less active in causing hemolysis of various mammalian erythrocytes, but, as indicated above, the mechanism of this phenomenon does not seem to be the same for all the bacteria or their products.

In the light of these demonstrations it was deemed advisable to determine if the organisms with which we are engaged in this investigation possess similar properties, and, if so, in what manner the phenomenon may be interpreted. For this purpose both the living bacteria and the filtered (sterile) products of their growth were allowed to remain in contact with the standard five per cent suspension of washed mammalian erythrocytes in 0.85 per cent NaCl solution under varying conditions of time and temperature.

The results were irregular; but, in general, it may be said that the erythrocytes of dogs, rabbits, guinea-pigs, and bovines were dissolved in varying degrees by both the living bacteria and the sterile filtered products of their growth. All of the five species of bacteria used by us when allowed to grow in the blood suspensions caused more or less escape

of hemoglobin from the corpuscles in a few hours. The hemolysis thus produced was always peculiar from the dark cherry-red color of the dissolved hemoglobin. This we take to be hemoglobin reduced by the growing bacteria, though we did not study it spectroscopically. As a rule, the color of the dissolved hemoglobin after hemolysis by the sterile filtrates from *B. subtilis*, *B. proteus* (vulg.), and *B. fluorescens* was of the bright red color commonly seen when, for instance, erythrocytes are dissolved by a hemolytic serum; but with filtrates from *B. prodigiosus* and *B. pyocyaneus*, the color was generally identical to that caused by the living bacteria. In these latter cases the reduction of hemoglobin, if the color seen be due to reduction, occurred without the intervention of living bacteria, for by culture tests our solutions were found to be sterile.*

On the other hand, the hemolytic activity of the filtrates free of living bacteria depended principally upon the age of the culture filtered. Except on dog's corpuscles, which seemed to be unusually sensitive to slight osmotic disturbances, our filtrates rarely exhibited hemolytic activity when the cultures were less than seventy-two hours old, and often they needed to be older than this before hemolysis was at all active.

We regard it as of special importance to note that the rate of hemolytic activity of the filtrates is in marked contrast to that of the bacteria themselves. When the blood suspensions were purposely inoculated with very small quantities of living bacteria, — from agar agar cultures, for instance, — hemolysis began within a few hours, sometimes long before bacterial growth in the inoculated suspension was certainly evident to the naked eye; whereas when the filtrates, free of living bacteria, as shown by culture tests, were employed, hemolysis occurred (with one exception) only with filtrates three or four days old. We regard this difference as explainable in the same manner as was explained the action of gelatin

* This difference between the action of bacteria and their filtrates has also been noted by Schur in his study of staphylolysins. Hoffmeister's Beiträge, 1902, Band iii., p. 89.

in inducing enzyme elaboration by the bacteria; *i.e.*, the erythrocytes with which the bacteria were in intimate contact stimulated in a specific way those atom-complexes of the bacterial protoplasms having affinity for the erythrocytes, and there resulted sufficient over-production of these atom groups to account for the prompt destruction of the erythrocytes and consequent liberation of their contained hemoglobin. On the other hand, the conditions of artificial cultivation under which the filtrates were prepared offered no such specific stimulus, so that while growing the bacteria elaborated this hemolyzing agent slowly and in only small amounts. This observation, together with that noted above in connection with the influence of gelatin in stimulating bacteria to the active production of liquefying enzymes we offer as contributory evidence to the theory advanced by Professor Welch in his Huxley lecture (1902), to the effect that certain functions of bacteria (parasites in general) are exhibited only under the influence of specific stimuli, and the singular differences often seen between the functions of bacteria in the test tube and in the living body are referable to the absence of such stimuli from the environment of artificial cultivation. Just as the somatic cells under the influence of a number of intoxicants become active in the multiplication of certain atom-complexes normally contained within them, "antibodies" or "receptors" as we call them, so by a reverse process may we conceive a like increase of particular complexes normally present in the bacterial or other living alien cells, to result from the stimulating influence of special integers resident within the tissues of the host. The "bacteriogenic cytotoxins" so elaborated are conceived by Professor Welch to be the real agents of tissue destruction in many types of infection, and to be the specific harmful products of many pathogenic bacteria from which it has as yet been impossible to isolate, under artificial conditions of growth, intoxicants that in either character or amount could reasonably account for the grave lesions that accompany their invasion of the susceptible body. Unless the elaboration of destructive poisons by such bacteria be conceived as

teleological, there seems to be but one explanation for it, viz., that it is the result of a specific stimulation of the bacteria by the tissues with which they are surrounded.

A number of experiments were made by mixing with the blood-suspension varying amounts of dead bacteria, *i.e.*, bacteria killed by chloroform and the latter driven off at the temperature of the thermostat. In no case did hemolysis result until the dead bacteria were employed in large amounts, four to five loops of a solid culture to one cubic centimeter of blood suspension; so that the hemolyzing agent is probably not present in the bacterial protoplasm to any great extent, though it obviously must be normally present.

The observations of Jordan¹² upon old filtrates from *Bacillus pyocyaneus* led him to regard their hemolytic activity as due to the alkaline reaction, or, more correctly, to the accumulation in the old pyocyaneus cultures used by him of hydroxyl ions. By neutralization or very slight acidification of such alkaline pyocyaneus filtrates their hemolytic activity was very much reduced or eliminated entirely. With the filtrates used by us we found essentially the same to be the case, though there were occasional irregularities and slight differences between the products from the several species. (See Table II.)

TABLE I.

Table Showing the Effects of Neutralization and Acidification upon the Hemolytic Activities of Bacterial Filtrate. The Tests were made by adding to 1 cc. of a 5 per cent Suspension of Washed Erythrocytes 0.5 cc. of the Several Filtrates.

HEMOLYSIS.

FILTRATE.	Reaction.	Dogs' Erythrocytes.		Bovine Erythrocytes.	
		After 2 hours at 37° C.	After 2 hours at 37° and 12 hours in ice chest.	After 2 hours at 37° C.	After 2 hrs. at 37° C. and 12 hours in ice chest.
B. fluorescens	16' Alkaline*	+	++	o	+
	Neutral	±	±	o	o
	10' Acid	o	+	+	+
B. pyocyaneus	14' Alkaline	++	+++	+	++
	Neutral	o	±	o	+
	10' Acid	o	±	o	±
B. prodigiosus	15' Alkaline	+	++	o	+
	Neutral	+	+	o	o
	15' Acid	o	o	o	±
B. proteus vulg.	10' Alkaline	++	+++	o	+
	Neutral	+	+	o	o
	10' Acid	o	o	o	±
B. subtilis	2.5 Alkaline	o	±	++	+++
	Neutral	o	±	++	+++
	10' Acid	o	±	++	+++

* 16' alkaline = Requiring 16 cc. normal acid to the liter to neutralize.

10' acid = Requiring 10 cc. normal acid to the liter to neutralize.

+++ = Complete hemolysis.

++ = Marked hemolysis.

+ = Slight hemolysis.

± = Doubtful hemolysis.

o = No hemolysis.

This series of tests was controlled by the exposure of red blood corpuscles from different animals to alkaline solutions $((\text{NH}_4)_2\text{CO}_3)$ of the same alkalinity as that of the filtrates.

The results were that ammonium carbonate in 0.85 per cent NaCl solution causes a slight degree of hemolysis with bovine, rabbit, and guinea-pigs' corpuscles, while with dog's corpuscles the effect is more marked. In this connection it is important to note that the filtrates from all the organisms used, except *B. subtilis*, caused hemolysis more readily with dog's erythrocytes than with those from rabbits, guinea-pigs and bovines; while the filtrate from *B. subtilis*, on the other hand, was much more hemolyzing for rabbit, guinea-pig, and bovine corpuscles than for those of the dog.

By heating, even to as high as 100° C. for from fifteen to thirty minutes, the hemolytic activity of the filtrates was unchanged, except in the case of the filtrate from *B. subtilis*. This organism, as well as filtrates from fluid cultures of it, differed, as a rule, in most respects from the others in the group, and in many particulars leads us to suspect that a closer study of it than is possible at this time would lead to interesting results. For instance, when *B. subtilis* was grown in blood suspensions hemolysis resulted much less promptly than was the case with the other organisms studied. On the other hand, the hemolysis resulting from the use of its filtrate was most active, and as a rule was demonstrable in filtrates from much younger cultures than was the case with the others. When heated to 56° C. for thirty minutes, the hemolyzing action of the filtrate from this organism sometimes disappeared, though at other times it was less sensitive to heat. For dog's corpuscles its hemolytic activity was regularly destroyed by the low temperature mentioned, while, as said, the other filtrates could usually be heated to a much higher temperature without their activity being destroyed.

When to a mixture of blood suspension and filtrate there was added normal rabbit, guinea-pig, or dog's serum the hemolyzing action of the filtrate was to some extent lessened, at times markedly so, but was not as a rule totally suppressed.

When heated to 56° C. for thirty minutes the restraining action of these sera was diminished to a large extent, but it

was not apparently totally suppressed; when heated for the same time to 70° C., it was to all appearances completely destroyed.

While all the normal sera had, as stated, some effect in restraining the hemolytic activity of the filtrates, this effect was always most marked when the serum and the erythrocytes were from the same species of animal; that is to say, dog's serum protected dog's corpuscles, rabbit's serum rabbit's corpuscles, guinea-pig's serum guinea-pig's corpuscles, more actively than was the case when the combinations were otherwise.

Such immune sera as were tested, *i.e.*, sera from rabbits repeatedly injected with three of the bacterial filtrates in large quantities, were as a rule a little more active in preventing hemolysis by their corresponding filtrates than were normal sera, and they were also a little more actively inhibitory for their corresponding filtrates than for the others used in the tests.

A number of efforts were made to determine if in the molecular structure of the hemolyzing agent there is comprehended a haptophore group having the power to bind it to the receptive erythrocyte. If such is, in fact, the case, it should be possible by the customary saturation method at low temperature to remove from the filtrates their hemolyzing properties, or at least to reduce them.

To decide the point mixtures of erythrocytes (dog's and rabbit's), and the several filtrates were kept for fourteen hours in the ice chest, at the end of which time the overlying fluid, consisting principally of filtrate, was tested for hemolytic action. In no case did we find a reduction after standing in contact with the corpuscles under the conditions named. Hemolysis was produced by the same amounts of the filtrates so treated, and in the same time as was the case with untreated filtrates. For instance:

To tubes containing 2 cc. of 5 per cent suspension of dog's corpuscles there was added 1 cc. of filtrate of *B. fluorescens*, *B. pyocyaneus*, *B. proteus vulgaris*, and *subtilis* respectively. This was placed on ice for twelve hours; 14 cc. of the clear supernatant liquid (containing approximately

0.5 cc. of filtrate) was removed and added to tubes containing 1 cc. of dog's corpuscles. Results as follows:

One cc. dog's corpuscles + treated fluorescens filtrate showed slight hemolysis in two hours, marked in twelve hours.

One cc. of dog's corpuscles + treated pyocyaneus filtrate showed marked hemolysis in two hours, complete in twelve hours.

One cc. of dog's corpuscles + treated prodigiosus filtrate showed slight hemolysis in two hours, marked in twelve hours.

One cc. of dog's corpuscles + treated proteus vulgaris filtrate showed no hemolysis in two hours, slight in twelve hours.

One cc. of dog's corpuscles + treated subtilis filtrate showed slight hemolysis in two hours, marked in twelve hours.

Controls 1 cc. of dog's corpuscles + 0.5 cc. of each of the above filtrates *untreated* and 1 cc. of 0.85 per cent. salt solution gave same results.

In short, we got no evidence that the molecular structure of our hemolyzing agent comprehended a haptophore group, capable of combining with red blood corpuscles under the conditions of our experiment.

We undertake the interpretation of these results with some hesitation. From the data presented, the escape of hemoglobin from the corpuscles seems to us to be explainable in one of two ways only, either as a result of osmotic disturbances caused by the chemical composition of the filtrates, or to the activity of the proteolytic enzymes in these filtrates upon the stroma of the erythrocytes, digesting it and thereby liberating the hemoglobin. We are inclined to the latter view.

While it is true that the hemolyzing action of the filtrates is lessened, or even at times eliminated, by neutralization or slight acidification, still this does not necessarily demonstrate that the hemolysis is due to the alkalinity of the filtrates, for we saw an analogous reduction of the gelatin-liquefying powers of these same filtrates by a similar neutralization and acidification.

Heating the filtrates, in some cases even to the boiling point, may lessen without entirely destroying their hemolytic activity. An analogue for this was noted above in connection with our studies of the proteolytic activity of these same filtrates as exhibited upon gelatin; and a further parallelism

between proteolysis and hemolysis is noticed in the different reactions of the several filtrates to heat; thus, for instance, we noticed that both the liquefying and hemolyzing activity of filtrates from *B. subtilis* and *B. pyocyaneus* were influenced more readily by high temperature than was the case with the other organisms in the series.

The reactions between the filtrates and normal and immune sera, while somewhat irregular, are, nevertheless, in general analogous in their relations to proteolysis and hemolysis.

From the data presented we believe that one may as reasonably attribute the hemolysis exerted by these filtrates to the action of their proteolytic enzymes upon the stroma of the erythrocytes as to any other factor.

The foregoing observations seem to warrant the following deductions:

1. That the destiny of non-toxic, though otherwise physiologically characteristic products of bacterial life in the body, is determined by the presence of specific neutralizing substances that can be demonstrated in the circulating blood.
2. That by the customary methods of artificial immunization the amount of such antidotal substances in the blood may be increased, but only to a slight degree.
3. That, through the use of sera from animals immunized from the non-toxic bacterial products, poor though such sera are in specific immune body, it is possible to distinguish the proteolytic enzymes resulting from the growth of different bacterial species from one another, as well as from certain physiologically analogous enzymes of animal origin.
4. That the proteolytic enzymes elaborated by certain bacteria in the course of their growth are much more resistant to high temperatures than is generally supposed; some being capable of exhibiting their characteristic function after exposure in the moist state to a temperature of 100° C. for fifteen to thirty minutes.
5. That the so-called hemolysins of bacterial origin are, at least in some cases, probably proteolytic enzymes.

6. That it is possible by experimental means to contribute material support to the doctrine of Welch concerning the origin of "bacteriogenic cytotoxins."

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AN EXPERIMENTAL STUDY OF THE BACTERIOLYTIC COMPLEMENT CONTENT OF THE BLOOD SERUM IN NORMAL, VACCINATED, AND VARIOLATED RABBITS.

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It was in accordance with the conception of the importance of the variations of the bacteriolytic complement content of the blood serum under certain conditions that the present study was undertaken, in connection with other experimental work in variola.

For these experiments rabbits were used for several reasons. Their blood serum was found to coincide very closely in germicidal power with human serum against the bacteria used. While rabbits suffer no clinical disturbances after small-pox inoculation, nevertheless certain definite reactions take place, *e.g.*, the corneal lesions after vaccination, and it was hoped that the index to systemic reaction, if such occurred, might be measured by the complement content, the main observation being the gross study of the quantity of the complement content before and after inoculation.

Investigations of this sort present certain difficulties, for not only is a pure complement not to be obtained, but also — in the case of this investigation — the specific nature of the contagium is unknown and complement change can only be estimated through an indirect mode.

Methods.—The blood of these experiments was obtained under aseptic precautions, either from the ear vein of the rabbit or from the vessels of the neck. If from the ear, the hair was clipped and the ear thickly smeared with a depilatory powder containing barium sulphide. The ear was washed subsequently with sterile salt solution to remove all traces of the powder, the vein distended by pressure from below, and an incision made into it with a sharp pointed pair of scissors. From this incision seven to ten cubic centimeters

of blood may be quickly obtained in a sterile test tube. When amounts of blood greater than ten cubic centimeters were desired, the animal was bled from the neck, the Latapie apparatus being used, the model for which was kindly furnished me by Dr. Pearce, of the University of Pennsylvania. Later, however, a simple method devised by Dr. Brinckerhoff was employed.

All of these methods gave parallel results. Control cultures were made, and, in all instances where the serum was used, were sterile. In no instance was the blood kept longer than twenty-four hours after being drawn, the animal being bled the day preceding the tests, and the blood allowed to separate in the ice-box over night.¹ The remainder of the technic is essentially the same as that used by Longcope² in estimating human complement.

Emulsions of the organisms used in these tests were made in sterile normal salt solutions from twenty-four-hour agar slants, the same strain of each organism being used. Such a number of bacteria were mixed with the salt solution as to produce not more than forty thousand colonies when a loopful of the mixture was plated for control. To a series of tubes, each containing one cubic centimeter of unheated blood serum, six loops of the salt solution containing the bacteria were added. A second series of tubes, each containing one cubic centimeter of serum, heated for one hour at 57° C. to destroy the complement, were next prepared and fresh (complement-containing) serum added in varying amounts; one-twentieth cubic centimeter for *B. typhi* and six-tenths cubic centimeter for *B. coli*, of fresh serum being the amounts found necessary to reactivate the heated serum. As with the first tubes, four loops of the bacteria mixture were added to each tube. From each tube of both series, which now contained blood serum plus bacteria, four loops were added to a tube of melted agar and plated immediately. The tubes

¹ Since writing this article E. W. Ainsley Walker (*Cent. f. Bakt. Orig.*, 1903, xxxiii, p. 297) has shown that there is a variation in the complement from hour to hour after the blood is shed. It may be stated, then, that the blood was withdrawn from eighteen to twenty-four hours before using, and was in contact with the clot during this time.

² Longcope. *Univ. of Pa. Med. Bull.*, 1902, Nov.

were at once put into a thermostat at 37° C., and at intervals of one, six, and twenty-four hours the plating process was repeated. All the plates were kept in the thermostat at 37° C. for twenty-four hours before the colonies were counted. Double sets of plates were made in many instances, and plants made from the same tube on different plates showed a remarkably accurate parallel. Quite notable also are many of the results as to the fact that normal rabbit serum causes a diminution in the number of typhoid colonies very nearly parallel to the diminution caused by human serum.

The first experiment was to determine the bacteriolytic power of the serum in normal rabbits and to see to what degree this varied. For this eight rabbits were used and, as was expected, some variations occurred. It was found, except in two instances, that one cubic centimeter of unheated active serum gave complete bacteriolysis for the typhoid bacillus in six hours. Of these two, one, starting with an immediate growth of twenty-two thousand colonies, showed two hundred and eighty colonies in six hours, the other, starting with an immediate growth of nine thousand six hundred colonies, showed three colonies in the six-hour plate. Both gave sterile plates in the twenty-four-hour plants. So that bacteriolysis was complete in twenty-four hours in all cases for *B. typhi* with one cubic centimeter of fresh serum.

When one cubic centimeter of heated serum was reactivated with one-twentieth cubic centimeter of fresh serum (complement) nearly similar results were obtained. The two sera that showed growth in the six-hour plates where one cubic centimeter of fresh serum was used also showed growth in the tubes where reactivation was attempted with one-twentieth cubic centimeter of the same serum. One other serum that gave sterile plates with one cubic centimeter in six hours showed six colonies in the one-twentieth cubic centimeter six-hour reactivated plate. Otherwise the results were parallel, showing that one-twentieth cubic centimeter of fresh serum was efficient in destroying the bacteria when supplied with sufficient amboceptors, as was one cubic centimeter of the whole serum.

For the colon bacillus a more considerable variation occurred: one cubic centimeter of serum destroying all the bacteria in the twenty-four-hour plates in only five instances; in three instances giving a growth of two hundred and sixty, twelve hundred and eighty, and sixty colonies respectively; immediate plants being seventeen thousand, seventeen thousand, and five thousand nine hundred colonies. In reactivating for *B. coli* six-tenths cubic centimeters of fresh serum was used, and this gave reactivation in only three of the five instances where sterile plates were obtained in twenty-four hours with one cubic centimeter of the fresh (unheated) serum. This seems to show that the reaction of the blood serum of the rabbit to the colon bacillus is not so uniform as it is to the typhoid organism, and that the latter is the better organism in general for observations of this sort. With smaller amounts of the bacteria, however, a more constant result would probably be obtained.

It may be stated that the sera that showed the most marked diminution in complement for *B. coli* were the same that showed the diminution for *B. typhi*.

As far as could be seen from subsequent observations the rabbits that showed this diminution of complement were in all respects normal.

The results of these observations on the normal rabbit show a marked germicidal power of the blood serum, although attended with some fluctuation, for the typhoid bacillus, which corresponds closely with the germicidal power of human blood serum for this same organism. The results with the colon bacillus are likewise similar, but apparently suffer more considerable fluctuation.

The second series of observations was conducted on vaccinated rabbits. The rabbits were vaccinated on the inner surface of the ear in two places. The H. K. Mulford Co. vaccine virus was used. The vaccination was performed aseptically, and the edges of the ear brought together and held in place in the manner described by Tyzzer. The rabbits suffer no clinical disturbance from vaccination, and

no variation in the bacteriolytic complement of the blood serum that is worthy of note was found on observations made on six rabbits.

Starting with an initial growth of from seven thousand to eighteen thousand colonies, all of the sera except one gave sterile six-hour plates with *B. typhi*, where one cubic centimeter of unheated serum was used. In this case there was a growth of nine colonies in the six-hour plate. All the twenty-four-hour plates were sterile. With one-twentieth cubic centimeter reactivation the serum that gave nine colonies in the six-hour plate gave twenty-five colonies in this six-hour plate, and three colonies in the twenty-four-hour plate. One other serum gave fifteen colonies in the six-hour plate. Otherwise the plates were parallel.

The colon plant showed much the same variation as did those in the unvaccinated rabbit.

The third series of observations were made on variolated rabbits. The vesicle contents, pustule contents, and disks — the inspissated contents of the dried lesion remaining between the layers of the skin in patients convalescing from small-pox — were used for inoculation. The vesicle and pustule vaccinations were made both on the corneas and ears, and where disks were used they were planted subcutaneously. As far as could be observed, the animals showed no clinical symptoms than these inoculations. No decrease in the bacteriolytic power of the blood serum was noted in these cases. The observations were made on four rabbits six days after variolation, as at that time the vaccine bodies have developed in the cornea, and the reaction of the blood should occur then, if at all. With rabbits, observations were made on the day of variolation, two days after variolation, and six days after variolation.

The immediate plants in this series average seven thousand colonies. The four rabbits bled six days after variolation gave sterile plates in six hours with *B. typhi* plus one cubic centimeter of fresh serum, except in one instance. In this case there was a growth of twenty colonies. The twenty-four-hour plates were all sterile. Reactivation of heated

serum with one-twentieth cubic centimeter of fresh serum likewise gave sterile plates for the typhoid bacillus in twenty-four hours in each case. With this reactivation there was, however, a growth of fifteen and one hundred and fifty colonies respectively in two of the six-hour plants, the latter being from the same rabbit that gave a growth of twenty colonies in the six-hour plants with one cubic centimeter of serum.

The rabbits which were bled on the day of variolation, two days after, and six days after gave sterile plates in twenty-four hours with one cubic centimeter of fresh serum, and with one-twentieth cubic centimeter reactivation. In the six-hour plates one plant from the third bleeding gave a growth of forty-two colonies with one cubic centimeter heated serum, and fifteen colonies with the one-twentieth cubic centimeter reactivation. The other six plates were sterile.

In this latter case there is, it is true, a slight diminution in the bactericidal power of the serum, but from the former observations one could not say it was due to the variolation. Several observations made on the same rabbit usually show these slight changes which may well come within the limits of error, or may be due to fluctuations which occur incident to the keeping of the animal. When one considers that a change may occur in the serum, such as to give an infinite growth in twenty-four-hour plates, one hesitates to lay too much stress on these lesser changes in the six-hour plates.

Observations were also carried on with the colon bacillus in this series, but as previously the results were too variable to be of any value.

Observations on one rabbit were made to see if any immediate reaction occurred in the blood serum in the course of the absorption of the small-pox contagion. For the inoculation a number of disks from virulent cases of small-pox were ground in a mortar, suspended in normal salt solution, and the emulsion injected, part into the ear vein and part into the peritoneal cavity of the animal. The rabbit's blood was drawn immediately after inoculation and at intervals of

thirty minutes, one hour, and three hours. The fresh defibrinated blood was used for these tests. The heated serum to which this blood was added for reactivation was from another rabbit, but previous experiment has shown us (as in the experiment of Longcope (*loc. cit.*) with human serum) that the interactivating power of normal serum does not vary. The results in this case show that no bacteriolytic diminution of the blood occurs immediately after inoculation — sterile plates being obtained in twenty-four hours in all cases for *B. typhi* with one-twentieth cubic centimeter reactivation. There was a growth of a few colonies in the six-hour plates at the first and third bleeding.

The complement content of two rabbits was also determined two weeks after variolation, and as in the previous cases no diminution was to be noted.

During the course of this work it was not infrequent to lose rabbits from various diseases which are incident to animals in confinement. Several animals at various times were taken when they appeared at the point of death from certain laboratory or induced diseases, chloroformed, the blood withdrawn, and the animal subsequently killed and autopsied. The results from four rabbits so examined show a remarkable and uniform decrease in the bacteriolytic complement of the serum.

Of these rabbits, one was affected with snuffles, and autopsy showed abscesses of the right lung and liver. The second rabbit was used when at the point of death from marasmus. The third rabbit showed colon septicemia, and the fourth leptothrix infection.

All of these rabbits gave a growth of from twenty thousand to innumerable colonies in the twenty-four-hour plates, and a correspondingly large growth in the six-hour plates for *B. typhi*, with one cubic centimeter of fresh serum and with one-twentieth cubic centimeter reactivation.

These latter observations are of interest as coinciding with the decrease in bacteriolytic complement found in individuals in the course of disease.

With a lack of more intimate knowledge at the present

day of the complexity of the forces involved in these reactions, we realize that work along the lines outlined must of necessity be of secondary interest. Especially in a disease such as variola, where the contagium is unknown, and where an indirect method must be used in approaching these problems, does this work offer discouragements. Nevertheless certain things may grow out of experiments of this kind. That the determination of the bacteriolytic complement content of the blood serum, even by indirect methods, is of importance, both in health and disease, is, however, apparent.

A method for determining the complement content of individuals at frequent intervals without discomfort to the patient and without too great loss of time to the operator would seem to make observations along this line of clinical as well as of scientific importance. In a later article a technic by means of which minimal amounts of serum may be used for these observations will be described.

CONCLUSIONS.

The bacteriolytic complement content of normal rabbit blood serum is closely allied to the bacteriolytic complement content of normal human blood serum for the typhoid and colon bacillus.

Normal rabbits show some individual variation in complement content for the typhoid bacillus, and more marked fluctuation for the colon bacillus.

Vaccination and variolation do not affect the bacteriolytic complement content of rabbit's serum for the typhoid and colon bacillus.

The blood serum of rabbits in the last stages of certain fatal diseases shows a marked diminution of bacteriolytic complement.

NOTE. — I wish to express my thanks to Dr. W. T. Councilman, at whose suggestion and under whose direction this work was carried on, and to Dr. W. R. Brinckerhoff for his kindness in supplying me with variolated rabbits.

THE BACTERIOLYTIC COMPLEMENT CONTENT OF THE BLOOD SERUM IN VARIOLA.¹

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In undertaking the study of the complement content in variola, one of the first difficulties that presented itself was to obtain sufficient amounts of blood from each patient to carry out the number of observations that it seemed necessary to make in the different stages of the disease. In an infectious disease of this sort, where secondary infections are so common, it seemed hardly justifiable to open, several times or even once, one of the larger veins, and moreover the extensive edema and distribution of the skin lesions in many of the cases would render the opening of an arm vein a rather dangerous and delicate surgical operation. While there are methods for determining the complement content of the blood with small amounts of serum, notably those of A. E. Wright² and Ainsley Walker,³ they seemed to us less satisfactory than the technic described by Longcope⁴ in his study of chronic disease. This latter, however, which seemed to be the most satisfactory in many ways, presented the difficulty of requiring considerable amounts of blood serum in the carrying out of its details.

A consideration of this method shows that the greater part of this serum is used after inactivation to supply sufficient amboceptor for the complement tests, and that in reality very minute amounts of the fresh patient's serum is necessary for the destruction of the *B. typhosus*, provided one has sufficient amboceptor. If, therefore, any means could be devised whereby an amboceptor other than that of the patient himself could be substituted that would link with the bacteria on

¹ Received for publication May 18, 1903.

² *Lancet*, 1900, 2, p. 1556.

³ *Centralbl. f. Bakt. Orig.*, 1903, 33, p. 297.
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the one hand and with the complementary body on the other, a method would be obtained whereby extended observations could be carried on in the course of a given disease because of the fact that only a very minute amount of the patient's serum would be required for the test.

Longcope (*loc. cit.*) himself showed that heated serum of different individuals could be substituted indifferently in reactivating a given complement, so that it made no difference whether a given complement was added to the heated blood serum of that same individual or to the heated blood serum of another individual in obtaining bacteriolysis. If, then, different human amboceptors would link with a given complement so as to give bacteriolysis, the thought suggested itself: Why might not certain animal sera contain an amboceptor that would fit the haptophore of a human complement and accomplish the same result?

Our own observations on the complement content of the rabbit showed a remarkable parallel between the reactivating power of rabbit and human sera. The experiments of Greenbaum and others on the precipitins show a close analogy between the blood of man and of monkey.

Therefore a series of experiments were undertaken to determine whether or not sera of other animals when deprived of complement could be substituted for heated human serum in supplying an amboceptor for human complement. The result of these preliminary experiments show conclusively that the amboceptors of at least three different blood sera, namely, those of the monkey, of the rabbit, and of the horse, will combine with the complement of human sera giving bacteriolysis for the typhoid bacillus in identically the same manner as when the heated and unheated serum of the same person is used for the test. Therefore complement estimates can be made as often as desired without danger or disturbance to the patient, as a simple puncture of the ear yields enough serum for this test.

An understanding of this phenomena may perhaps be aided by enlarging upon the simile of Fischer, who likens the cell or bacterium to a lock, the amboceptor to a key, and the complement to the hand that turns the

key. Bordet showed that the heated serum (amboceptor) could be reactivated so as to produce hemolysis by the addition of complement from various sources. In other words, given the lock and key, almost any hand could turn the key. On the other hand, the fact that several different keys might fit the same lock had not been given consideration. But that such is the case our experiments show. The only thing being necessary is to have keys of very similar construction, and in the case of human and some of the animal serum the similarity is so near that the lock may be turned by either of these keys, provided there is strength enough in the hand (complement) to manipulate the key. To complete this analogy, bringing in the importance of a deficiency of complement as a cause of disease, we may say that the hand is not strong enough to turn the key, and thereby the door remains unlocked. Such may be the case in certain individuals; there is a deficiency of complement and, though we have the lock and key, immunity fails to close the door, and the individual suffers the results of the invasion of a pathogenic organism.

In following this analogy, however, it is well to remember that the importance of only one of the factors concerned in bacteriolysis is emphasized, and that in infectious processes we have many things other than the complement of the blood to consider. The complement content is, however, of marked importance, and a deficiency of the same, we may say in the light of our present knowledge, cannot exist without some danger to the individual.

Technic. — In the beginning of these experiments rabbit serum was used for reactivation, as it could be easily obtained; the blood of one large rabbit being sufficient for a considerable number of observations. Later on horse serum was substituted for the rabbit's serum, as it was found to answer the purpose and saved the time necessary for obtaining rabbit's blood. Normal horse serum was kindly furnished me through the courtesy of Dr. Kinyoun. This facilitated the work considerably. A point in regard to the horse serum is that it was found necessary to heat it for one and one-half hours at 57° – 58° C. to completely destroy the complement. It might also be stated that in inactivating a large number of tubes of serum at the same time they should not be closely packed, but should be so separated as to assure the full force of the heat reaching each tube immediately on being placed in the oven; otherwise tubes may be found which will give sterile plates without reactivation.

The steps of the process of the complement determination were as follows:

1. Obtaining rabbit's serum. The animal is chloroformed and the carotid exposed under aseptic precautions. The artery is then ligatured above, a sterile capillary tube connected with a sterile flask inserted into the artery, the clip removed and the blood allowed to flow into the flask. The blood is placed in a cool place until the serum has separated. When the separation is complete, the serum is transferred by means of a sterile pipette into small tubes one cubic centimeter to each tube. The tubes are then placed in an incubator at 57° C. for one hour to destroy the complement. The serum is now ready for use, or it may be kept for weeks, if necessary, in a cool place.

2. Obtaining patient's blood and reactivating rabbit's serum.—The blood for the complement observation is obtained from the patient's ear in the same manner as is generally used in procuring blood for a Widal test. Sufficient blood may be obtained by a single puncture, as one-fourth cubic centimeter is ample amount for a test. Tubes for this may be readily made from glass tubing. They should be sterile and plugged with cotton. The serum separates quickly, especially if a sterile platinum wire be run between the clot and the glass. When the serum has separated, the necessary amount (one-twentieth to one-tenth cubic centimeter) is transferred by means of a sterile capillary pipette into the tube containing one cubic centimeter of inactivated rabbit's serum. The serum thus obtained was sometimes used immediately on its separation from the clot, *i.e.*, about one hour after withdrawal, or was withdrawn at night and used the next morning, *i.e.*, twelve to eighteen hours after withdrawal. Walker (*loc. cit.*) has stated that a slight fluctuation in the complement is incident to the time the serum is used after withdrawal. These slight fluctuations which he gets in the complement content from hour to hour are very different from the marked changes observed in the course of investigations of disease, and would not be notable in an investigation of this

sort, where changes when they occur are much more marked.

Controls were made between the serum used immediately after withdrawal and the serum which had stood for twelve to eighteen hours, and no differences that would affect the general results here given were observed.

3. Preparation of typhoid emulsion.—The typhoid emulsion is prepared by adding to a tube containing three cubic centimeters of sterile (.85 per cent) salt solution a small amount of the growth from a twenty-four-hour streak culture of *B. typhosus* on agar or Loeffler's blood serum. The amount used is necessarily not always the same, but such an amount that one loopful of the mixture (salt solution + *B. typhosus*) will give a plate of from 10,000 to 30,000 colonies can be uniformly obtained. A double loop was used in transferring this mixture to the reactivated rabbit serum — three double loopfuls (approximately one-fiftieth cubic centimeter) — being added to the serum.

4. Plating.—Having now the preparation of amboceptor (heated rabbit serum) plus complement (patient's serum) plus *B. typhosus* emulsion, the mixture should be thoroughly shaken and two double loopfuls (or four single loopfuls) added to the agar and plated. The agar should be very thoroughly shaken before plating to insure a uniform growth of colonies. The tubes and plates are then placed in the incubator 37.5° C. and new plates are made at the desired intervals.

5. Counting colonies.—The exact estimation of the colonies in the plate where there is a considerable growth is not of particular importance, as when the colonies run into the thousands a few hundred one way or another make no particular difference, but when there is a scant growth showing that more or less bacteriolysis has taken place they should be counted carefully. The colonies were estimated in the cases where there was considerable growth by counting several square centimeters at different parts of the plate, and from them making an average of the number of colonies on the whole surface. Where there was a scant growth the

entire surface of the plate was counted. A No. 3 ocular was used to facilitate the counting process.

Having in mind the idea previously outlined, the first observation was to see if bacteriolysis could be obtained for *B. typhosus* by reactivating one cubic centimeter of heated rabbit serum with $\frac{1}{20}$ to $\frac{1}{10}$ cubic centimeter of fresh serum from a normal individual. Such was found to be the case, bacteriolysis being complete in six hours with both these reactivations on two healthy individuals from whom Widal amounts of blood were obtained for the test. While this was not conclusive evidence that the rabbit's serum furnished an amboceptor parallel to the human serum, nevertheless it pointed to such being the fact, and so the following test was performed :

R. T. Healthy male, bled from arm (25 cc.). Serum separated from clot twelve hours after withdrawal and pipetted into tubes, one cubic centimeter in each tube. These tubes placed in the incubator, at 57° C. for one hour. Tubes containing one cubic centimeter of freshly drawn rabbit's serum (A) and rabbit's serum (B) two weeks old that had been kept in a cool place were inactivated at the same time. These tubes were then reactivated with $\frac{1}{20}$ — $\frac{1}{10}$ cc. fresh, unheated (R. T.) serum, and an equal quantity of suspension of *B. typhosus* added to each. The control plant of one loopful of this suspension gave a growth of 30,000 colonies.

The results of this experiment show an accurate parallel between the tubes, whether heated human or rabbit serum was used for the intermediary body as may be seen from the following :

(R. T.)	Imme- diate.	6 hours.	9 hours.	24 hours.
1 cc. heated human serum plus $\frac{1}{8}$ fresh human plus B. typhi	12,000	Sterile	Sterile	Sterile
1 cc. heated human serum plus $\frac{1}{16}$ fresh human plus B. typhi	9,600	"	"	"
1 cc. heated rabbit serum A plus $\frac{1}{8}$ fresh human plus B. typhi.....	13,200	"	"	"
1 cc. heated rabbit serum A plus $\frac{1}{16}$ fresh human plus B. typhi.....	8,400	"	"	"
1 cc. heated rabbit serum B plus $\frac{1}{8}$ fresh human plus B. typhi.....	6,000	"	"	"
1 cc. heated rabbit serum B plus $\frac{1}{16}$ fresh human plus B. typhi....	12,000	"	"	"
1 cc. unheated human serum plus B. typhi	15,000	"	"
1 cc. unheated rabbit serum plus B. typhi	15,600	"	"
1 cc. heated human serum plus B. typhi	12,000	30,000	Innumerable
1 cc. heated rabbit serum plus B. typhi.....	14,000	32,000	Innumerable

The test was carried out in the same manner, substituting heated monkey serum (Rhœsus), and heated horse serum, in place of the heated rabbit serum. The results were the same as in the first experiment. Either of these latter sera serving to replace the heated human serum of the individual tested, and giving sterile plates in six and twenty-four hours when reactivated with $\frac{1}{8}$ cc. of the normal human complement containing serum. That this destruction of the bacteria would not take place in the heated animal serum alone was shown by the fact that when plants were made from these tubes of heated serum before reactivating with fresh human serum there was always a growth of from 30,000 to infinity colonies in the twenty-four-hour plates.

Having shown, then, that these heated sera could be used in quantitating the complement of an individual, a series of

observations were made on apparently healthy individuals to see what variations might exist. Heated rabbit serum was used for the intermediary body. It has already been shown by Wright (loc. cit.) and by Longcope (loc. cit.) that there is a considerable variation in the complement content in normal people depending, in their cases, especially upon overwork and anxiety. These observations bear out these facts, and show that in individuals who may in no way be called diseased a considerable diminution in the complement content may occur, which can at times be accounted for, but sometimes not.

These observations were made on twelve healthy individuals about the hospital, including physicians, nurses, and laborers. Of these, seven showed a high complement content at each observation, several observations being made on each during a period extending over six weeks, all giving sterile plates in six hours and twenty-four hours, with $\frac{1}{20}$ cc. of serum.

In two other cases the observations were of interest. In the first of these, both $\frac{1}{20}$ cc. and $\frac{1}{10}$ cc., inactivation failed to give sterile plates, even in the twenty-four-hour plants. The man on whom the observations were made had been doing exacting work for some time, with loss of sleep, and loss of air and exercise. Two days after the observation was made, two small streptococcus infections appeared on this man's hand. With a relief from work and the healing of the infections it was found that $\frac{1}{20}$ cc. of his serum gave sterile plates in six hours. The other of these cases had shown high complement on several observations, $\frac{1}{20}$ cc. giving sterile plates in six hours, but during the course of an attack of influenza the complement was found diminished, $\frac{1}{20}$ cc. of his serum failing to give sterile plates in twenty-four hours.

Of the three remaining cases of this group, one observation was made in two cases, the first, a girl, aged five years, who failed to give sterile plates in twenty-four hours, when either $\frac{1}{20}$ cc. or $\frac{1}{10}$ cc. of her serum was used for reactivation. Though in good health at the time, she had had an

attack of broncho pneumonia several weeks previously. The second of these cases, a male, middle aged, failed to give sterile plates, with $\frac{1}{20}$ cc. or $\frac{1}{10}$ cc. of his serum. The last of this group was a young, healthy Irish girl, on whom a number of observations were made, but in no case was such reactivation obtained, with $\frac{1}{20}$ or $\frac{1}{10}$ cc. of her serum, as to give sterile plates in twenty-four hours. In this latter case especially, no apparent reason presents itself for the diminution of the complement, and we must conclude, therefore, that in certain individuals this complement diminution exists. It makes it necessary, then, in studying any given disease, to take the possibility of such conditions into account before drawing too definite conclusions as to the disease being always the cause of the diminution of complement.

Assuming, then, from the above observations, that in the greater number of normal individuals $\frac{1}{20}$ cubic centimeters of their fresh serum would reactivate one cubic centimeter of heated rabbit serum so as to give sterile plates in from six to twenty-four hours, a series of observations were undertaken in patients afflicted with variola to see what changes, if any, might occur in the course of this disease.

It is to be understood that in studying a disease like variola, unless the epidemic be unusually severe, one cannot pick material, but must use whatever he is fortunate to find at his disposal at the time. In this investigation a fairly representative group of cases of the different forms and different degrees of severity of the disease came under observation. In all fifteen cases were studied and may be grouped as follows:

1. Variola vera — abortive type. Majority of lesions not coming to pustulation, six.
2. Variola vera — mild, but disease running complete course, five.
3. Variola confluens — all with more or less secondary infection, three.
4. Purpura variolosa, one.

The results of the observations were as follows :

I. — M., age 39. Variola vera (abortive). Moderate papular eruption on face, few lesions on trunk and extremities. Only a few lesions advanced to pustular stage. No constitutional disturbance. In hospital ten days.

First observation early pustular stage :

	Immediate.	24 hours.
1/20 reactivation.....	9,200	5

Second observation ; beginning dessication :

1/20 reactivation ..	12,000	Sterile
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Third observation, day of discharge :

1/20 reactivation	8,000	Sterile
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II. — F., age 32, Variola vera (abortive). Moderate papular eruption confined chiefly to face. No constitutional symptoms.

First observation contaminated.

Second observation, dessication began :

	Immediate.	8 hours.	24 hours.
1/20 reactivation.....	3,600	Sterile	Sterile

III. — F., age 35. Variola vera (abortive). Moderate general papular eruption. No constitutional symptoms after initial stage. In hospital ten days.

One observation only on this case and that at end of convalescence :

	Immediate.	24 hours.
1/20 reactivation	9,600	Sterile
1/10 "	12,000	"

IV. — F., age 38. Variola vera (abortive). Moderate general papular eruption. Only a few lesions advancing to pustular stage. No constitutional disturbance after initial symptoms. In hospital ten days.

Observations only five days before discharge. Dessication not complete at this time, but progressing finely.

	Immediate.	6 hours.	24 hours.
1/20 reactivation.....	8,000	14,600	12,000
1/10 "	8,400	3,600	1,200

V. — M., age 30. Variola vera (abortive). Moderate papular eruption ; abortive. No constitutional disturbance. Discharged well five days after entrance.

First observation day after entrance, late papular stage, some of the lesions beginning involution.

	Immediate.	6 hours.	24 hours.
1/20 reactivation.....	30,000	1,200	400
1/10 "	28,000	62	Sterile

Considering the large number of colonies in the immediate plant (30,000) the complement may be called within normal limits as $\frac{1}{20}$ cubic centimeter of serum from a normal person is not apt to give reactivation so as to obtain sterile plates in all instances with so high a number of bacteria.

Second observation, day before discharge — patient practically clear :

	Immediate.	6 hours.	24 hours.
1/20 reactivation.....	10,000	Sterile	Sterile
1/10 "	10,000	"	"

VI. — F., age 6. Variola vera (abortive). Entered hospital fifth day of disease showing papules, discrete, but rather closely packed over face, trunk, and extremities. Case passed through vesicular stage; some of the vesicles going on to papules, but more drying up. Temperature normal fourth day after entrance. Discharged well twelve days after entrance.

First observation, vesicular stage :

	Immediate.	6 hours.	24 hours.
1/20 reactivation.....	8,000	Sterile	Sterile
Second observation :			
1/20 reactivation	18,000	50	Sterile
Third observation, beginning dessi- cation :			
1/20 reactivation.....	12,000	Sterile	Sterile

VII. — M., age 4. Variola vera (mild). Entered hospital fourth day of disease. Early papular on face, body, and extremities. Disease advanced through distinct pustular stage. No complications, and dessication rapid. Temperature normal seventh day after entrance. Discharged well twelve days after entrance.

First observation vesicular stage.

	Immediate.	6 hours.	24 hours.
1/20 reactivation	7,200	4,000	800
Second observation, pustular stage :			
1/20 reactivation.. ..	20,000	200	Sterile
Third observation, beginning dessication :			
1/20 reactivation	18,000	50	Sterile

VIII. — F., age 65. Variola vera. On entrance marked papular eruption with diffuse redness of face. Trunk and extremities somewhat involved. Disease followed typical course, but without secondary infection.

First observation day after entrance, vesicular stage :

	Immediate.	6 hours.	24 hours.
1/20 reactivation	6,000	14,400	7,000
Second observation, pustular stage :			
1/20 reactivation	7,200	4,000	1,200
Third observation, beginning dessication :			
	8,000	—	900

This is the case of an elderly, hard-working woman who might well have shown a complement reduction irrespective of the disease. There is a small increase of complement in convalescence as compared with the first observation, but not a rise to normal limits.

In this case and in case IV. we have women who are somewhat run down and in whom we might expect a slower convalescence.

IX. — F., age 34. Variola vera (mild). Showed general papular eruption on entrance. Usual course. Pustular stage not very severe. Dessication fairly rapid. No secondary skin infections. No pitting. Temperature normal on sixth day after entrance. In hospital eleven days.

First observation, early papular stage :

	Immediate.	24 hours.
1/20 reactivation	12,000	1,800
Second observation, beginning dessication :		
1/20 reactivation	8,000	Sterile
Third observation day before discharge :		
1/20 reactivation.....	12,000	Sterile

X. — F., age 56. Variola vera (mild). Showed general papular eruption on entrance. Usual course. Pustular stage well marked. No secondary skin infection. No pitting. Temperature normal on sixth day after entrance. In hospital fifteen days.

First observation, early pustular stage :

	Immediate.	24 hours.
1/20 reactivation	6,000	9,600
Second observation beginning dessication :		
1/20 reactivation	7,200	120
Third observation, convalescence well advanced :		
	8,000	Sterile

XI. — M., age 36 years. Variola vera. Entered hospital on eighth day of disease. At that time showing discrete pustules, not numerous, on face, body, and extremities. Pustular stage at height day after entrance, after which dessication was rapid, with no secondary infection. No constitutional disturbance. Discharged well ten days after entrance.

Observations were made every second day until his discharge :

	Immediate.	6 hours.	24 hours.
1 1/20 reactivation.....	9,600	2,100	800
2 " "	12,000	150	35
3 " "	12,000	Sterile	Sterile
4 " "	22,000	120	"
5 " "	8,400	Sterile	"

This case shows a diminution of complement at first followed by a rapid rise beginning with the clearing up of the lesions. The protective power

of the serum in convalescence is well shown in that two days before discharge one-twentieth cubic centimeter was sufficient to give sterile plates in twenty-four hours from an initial plant of 22,000 colonies.

XII. — M., age 18. Variola confluens. Severe case. General distribution. Confluent on face. Marked edema. Severe superficial skin infections on face. Deeper furuncles on lower extremities. In hospital twenty-one days.

First observation sixth day in hospital, pustular stage :

	Immediate.	6 hours.	24 hours.
1/20 reactivation	9,000	16,000	30,000
1/10 "	7,200	1,200	4,500
Second observation, lesion showing dessication. Two furuncles.			
1/20 reactivation	8,000	—	Innumerable
1/10 "	9,200	—	20,000

Third observation, patient convalescing, boils practically healed :

	Immediate.	6 hours.	24 hours.
1/20 reactivation	4,200	—	Innumerable
1/10 "	6,000	—	9,600
Fourth observation, day of discharge. 12,000 — 6,000			
1/10 reactivation	12,000	—	Sterile

XIII. — F., age 10. Variola confluens. Rather severe case, general distribution of lesions, some confluent on face. Case marked by extensive secondary superficial skin infections over face and extremities which were very slow in clearing up. Considerable constitutional disturbance. In hospital thirty-one days.

Two observations made. Both near end of convalescence, four days and one day respectively before discharge. At this time there were a few superficial skin lesions that showed streptococcus in culture.

	Immediate.	6 hours.	24 hours.
27th day { 1/20 reactivation.....	3,600	4,800	14,000
{ 1/10 "	3,200	4,000	12,200
30th day { 1/20 "	7,000	12,000	22,000
{ 1/10 "	8,200	9,000	14,000

XIV. — M., age 11. Variola confluens. Severe case. General disturbance. Lesions became confluent over face. Rather severe throat symptoms exhibited. Highest temperature 103° F. Temperature regained normal on twelfth day of disease. Pitting about chin. In hospital fifteen days. Slight secondary infection.

First observation made near end of pustular stage :

	Immediate.	24 hours.
1/20 reactivation.....	6,000	30,000
1/10 "	6,000	9,000

Second observation day before discharge, patient practically well :

	Immediate.	24 hours.
1/20 reactivation.....	8,000	20,000

XV. — F., age 28. Purpura variolosa. Case of primary hemorrhagic small-pox. Entered hospital second day of disease. Face, body, and extremities brick red in color, subconjunctival ecchymoses, hemorrhages from mucous membranes. Died forty-eight hours after entrance.

A slight needle prick in ear caused a constant flow of blood which ceased only by the application of artery forceps.

First bleeding thirty-six hours before death.

	Immediate.	9 hours.	24 hours.
1/20 reactivation.....	14,000	30,000	10,000
1/10 "	12,000	1,200	900

While this showed a considerable diminution of complement as compared with the normal, nevertheless there was some bacteriolysis with 1/10 cc. reactivation.

Second observation twenty-four hours before death :

	Immediate.	9 hours.	24 hours.
1/5 cc. reactivation	7,200	Strep.	Strep.
			(No typhi.)

This observation was made with a larger amount of the patient's blood (1/5 cc.), and while bacteriolysis for *B. typhosus* was shown, the amount was sufficient for the cultivation of the streptococcus which was present in the blood, but which was not cultivated from the smaller amount of serum previously obtained.

Third observation nine hours before death :

	Immediate.	4 hours.	8 hours.	24 hours.
1/20 reactivation.. ..	3,200	4,800	12,000	32,000
1/10 "	3,200	4,200	12,000	30,000

For control, plants were made at the same time from a tube of heated serum plus *B. typhosus* not reactivated, and from a loop of heated serum plus *B. typhosus* reactivated with 1/20 cc. from a case of convalescing variola. These gave the following results :

One cc. heated serum plus *B. typhi* (no reactivation) :

	Immediate.	4 hours.	8 hours.	24 hours.
	3,600	4,800	12,000	30,000

One cc. heated serum plus 1/20 cc. fresh serum (conv. variola) plus *B. typhi*.

	Immediate.	4 hours.	8 hours.	24 hours.
	3,600	4	Sterile	Sterile

This shows a complete loss of complement in the hemorrhagic case. The plates forming an accurate parallel to the plates from the tube containing only heated serum. The convalescing case, on the other hand,

offers a notable difference under the same conditions, sterile plates being obtained in eight hours with a destruction of all but four colonies in four hours against 4,200 colonies in the hemorrhagic case.

Blood of the hemorrhagic case examined after death showed innumerable colonies of both streptococci and typhi.

From work that has previously been done and which is confirmed by these observations we know that

1. Most normal individuals have a high complement content — one-twentieth cubic centimeter of their blood serum added to one cubic centimeter of heated serum being sufficient to destroy large numbers of typhoid bacilli in a few hours.
2. That this complement content is remarkably fluctuating; conditions of lowered vitality due either to fatigue or infection may temporarily cause a diminution which may under proper conditions be promptly restored.
3. That certain individuals show on repeated examination a low complement content, the cause of which may not be evident.

Bearing in mind these observations on the normal individual we may next consider what relation the changes observed in the course of an acute infectious disease — variola — may bear to the factors involved in the causation and course of this disease.

An examination of the above tables will show that in all instances where an observation of the patient's blood was made early in the course of the disease a low complement content was found. The nearest approach to an exception to this rule is in case VI., where the first observation made on the fifth day of the disease showed a normal complement.

In the abortive cases and the cases running through the entire course of the disease, but unattended with secondary infection, there is a rapid return of the complement to the normal standard. This rise of complement content in the abortive cases is in coincidence with the beginning involution of the lesions. In the cases running through pustulation and unattended with secondary infection this rise may be noted in the later pustular stage or during the early stage of

dessication. One may, perhaps, say in some cases (see VII.) that an absence of secondary infection may be predicted when one gets a return of the complement to normal in the early pustular stage; but a prediction that secondary infection would occur could not be made at this time from a low complement, for in some of the mild cases (IV. and VIII.) complement may be low throughout the entire course of the disease just as it may be low in certain normal individuals.

Two cases (VI., VII.) offer an interesting comparison as regards the complement content reduction being in inverse ratio to the severity of the disease. Two patients, a boy and a girl, both infected from the same case, entered the hospital at the same time. At the time of their entrance, and until the vesicular stage was well developed, there was practically no difference to be observed in the severity of the two cases. The girl, however, did not go on to the pustular stage. Her lesions aborted and she quickly returned to health. Her complement was normal throughout.

The boy, on the other hand, continued on through a well-marked pustular stage, though without secondary infection, his case being considerably more severe and more prolonged than that of the girl. His complement showed a reduction until the late pustular stage, when just before involution of the pustules it rose to normal and so remained.

In the cases attended with secondary infection (from which streptococci were isolated) there was always a low complement content — the diminution continuing throughout convalescence in all of the observed cases, and the patient being discharged while the complement content was still low. In one (XII.) there was some increase in complement content at the last observation, though not a complete return to our arbitrary normal standard.

That there should be rapid rise of complement in the uncomplicated cases of variola seems natural from the resistance that the individual offers to this disease. In variola the recuperation of the patient is remarkable. With the passing of the lesion the patient in most cases is well in all senses of the word, and that a normal complement should accompany this quick return to health is not remarkable.

That a low complement content should persist when secondary infection occurs is of considerable interest, in that it bears out the work previously done in showing that a low complement is coincident with secondary infection (Longcope, loc. cit.). In this connection the case of hemorrhagic small-pox is of value in establishing the conclusion that a low complement is coincident with terminal infection.

As will be seen by examination of the table (XV.) this case suffered a progressive complement diminution from the time of her entrance to the hospital until her death.

To briefly summarize these observations, then, we find in variola:

1. A diminution of complement content in the early stage of the disease.
2. A rapid return of the complement content to normal in the cases unattended with secondary infection. This return is seen in the abortive cases with the beginning of the involution of the lesions; in the others, during the late pustular or early desiccating stage.
3. A failure of the complement content in its return to the normal in cases in which there is secondary infection.
4. A progressive continued diminution in the complement content when the case is complicated by terminal septicemia.

An interpretation of these results may be aided by a brief consideration of the disease, variola. In variola we have a clinical entity which involved two processes. (1.) Infection by a definite specific contagion. (2.) A secondary local or general pyogenic process, which may or may not be present, but which, if present, may offer the gravest consequences to the patient.

From our observations we must conclude: That infection with the specific contagion of variola causes a diminution of the bacteriolytic complement of the individual, and that this diminution is more or less dependent upon the severity of the attack. Also that a mild infection, in which there is very

little clinical disturbance, might occur without such complement diminution being noticed. This should be inferred from the fact that in one of our mild cases no diminution was present at the time the patient entered the hospital.

Now, given an individual attacked by a definite specific contagion, it is reasonable to suppose that this individual will exert all possible resistance against the contagion. Provided this resistance is of avail, it is shown by a rapid return of the complement to the normal standard. Provided, however, the resistance of the individual is for some reason weak or the infection is unusually severe, this rise of complement to normal does not take place and we have a patient entering the pustular stage of variola with a diminution of the bacteriolytic power of the blood serum.

Given then a lowered complement and a supply of pyogenic organisms (streptococci were isolated from the lesions of practically all of our cases), it is easy to see how variola may be complicated by a local or general pyogenic process, and from the fact that the complications due to pyogenic infections, and that death from streptococcus septicemia were, in the cases observed, always accompanied by a continued lowered complement content, we must conclude that this lowered complement content in variola is the cause of such infection taking place and is of danger to the individual.

The foregoing observations emphasize the fact that systematic studies of the bacteriolytic power of the blood serum carried out in various diseases would be of value. Especially does this seem to be true from a prognostic standpoint. The rapid advances that are now being made both in the technic and the understanding of the phenomenon of bacteriolysis should make such observations of practical importance.

NOTE.—I wish to express my thanks to Dr. W. T. Councilman, at whose suggestion and under whose direction this work was carried on, and to my associates at the Detention Hospital for their kindness and assistance.

THE NON-IDENTITY OF AGGLUTININS ACTING UPON THE
FLAGELLA AND UPON THE BODY OF BACTERIA.¹

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The relation of flagella to agglutination has received more or less attention since Malvoz's experiments published in 1897.² He found that typhoid bacilli retained on the Chamberland filter after prolonged washing no longer became agglutinated by typhoid serum or by certain chemical substances such as formalin and safranin. The bacilli acted on were no longer motile and cilia were not demonstrable. The subject was taken up by Dineur,³ who, after a painstaking series of experiments with several cultures of the typhoid bacillus, came to the conclusion that the cilia are the primary agents in the process and that the bacilli become attached to one another by means of these cilia. He goes over Malvoz's experiments and finds that the agglutinability is largely lost after processes of manipulation which injure or tear off the cilia.

H. C. Ernst and Robey⁴ have gone over the experiments of Dineur, but they conclude that the "agglutinating property does not lie in and is in no way connected with the flagella of the bacteria concerned." Nicolle and Trenel⁵ have recently reported observations bearing upon the same subject. They describe races of typhoid bacilli from water which failed at first to respond to typhoid agglutinin, but did so later after cultivation. They note a parallelism between motility and agglutinability. They make a distinction between

¹ Read at the Third Annual Meeting of the American Association of Pathologists and Bacteriologists, Washington, D.C., May, 1903.

² *Annal. de l'Inst. Pasteur*, 1897, no. 6.

³ *Bulletin de l'Académie Royale de Médecine de Belgique*, 1898, iv. series, Vol. xii, p. 705.

⁴ *Trans. Congress Amer. Phys. and Surgeons*, 1900, p. 26.

⁵ *Annal. de l'Institut. Pasteur*, 1902, xvi, p. 562.

"aptitude agglutinative" and "fonction agglutinogène," and infer from their own experiments that a bacillus which has been made non-motile at 42° C. does not produce agglutinin in animals. In view of the results stated by us below, that it takes ten to twenty times the amount of immune serum producing agglutination of motile bacilli to produce agglutination in non-motile forms, it is highly probable that they did not treat their animals long enough to produce the necessary agglutinin. This explanation is strengthened by the fact that the amount of culture material injected by them is relatively small. These authors are inclined to ascribe to the cilia "le rôle capital dans le phénomène de l'agglutination."

Defalle⁶ deals with the same subject with special reference to the capsules of the Friedländer and allied bacilli. He places great importance upon flagella in agglutination, but adduces no new facts.

The difficulty which is encountered at once in applying the theory that the cilia or flagella of bacteria are the chief actors in the process is that bacteria which possess no cilia are clumped in immune sera, but the agglutination appears only in dilutions much more concentrated than are necessary for the clumping of motile bacteria. The apparent contradiction between the conclusions of Malvoz and his followers and the agglutinability of non-motile forms is cleared up by the following observations which demonstrate the existence of flagellar as well as body agglutinins distinct from each other.

The following experiments were not made at the outset to determine the relation of flagella to the phenomenon of agglutination, but the facts to be reported were discovered quite accidentally in the course of a study of bacilli belonging to the hog-cholera group, with special reference to their agglutinative relationship. This study is published in the *Journal of Medical Research* for 1903, IX, p. 270, and for all details of the cultures employed and the methods used

⁶ *Annal. de l'Institut Pasteur*, 1902, xvi, 595.

we must refer the reader to that publication.¹ One of the cultures included in that group was considered of sufficient importance to deserve special consideration. This was described several years ago by one of us,² and it has been under observation more or less ever since.

It is well known that hog-cholera bacilli are actively motile. Their motility is much more pronounced than that of *B. coli*, for it may be observed in any culture medium and in cultures many days old. Moreover, the motility is general, nearly all bacilli sharing in it, and it does not decline materially as the period of cultivation becomes longer. The culture above referred to differs from the defined type α in that the bacilli are non-motile. In all other respects it is identical with the motile type. Motion has never been seen during the years of use in this laboratory.

When the studies on the agglutinative characters of the hog-cholera group were begun by one of us in 1898, the intimate relation between the motile and the non-motile type was soon made evident, and mentioned in the article quoted. During the past year a more detailed investigation of this relationship was made, which has revealed certain important facts destined to explain hitherto controversial views, and to illustrate the complex nature of agglutination phenomena already suspected by many who have devoted time to this subject.

The action of the serum of rabbits and guinea-pigs immunized with the non-motile bacillus upon this bacillus differs in several particulars from the action of serum produced by motile bacilli on the latter.

1. The clumping appears at least several hours later.
2. The clumps appear in the form of a uniform precipitate of compact granules, at first excessively small and later

¹ As a supplementary note to the observations reported in that paper, it should be stated that the members of the group there studied act alike upon mannite, and in the same way as they act upon dextrose. This medium does not, therefore, distinguish between the individuals. It should also be stated that a culture of *B. icteroides* recently received through the kindness of Dr. Flexner, directly from Dr. Sanarelli shows the same agglutinative affinities toward the hog-cholera bacillus α as does the culture from Dr. Reed, used in the published tests.

² Centralblatt für Bakteriologie, 1899, xxv, 241.

growing larger, if the serum is of the proper strength. Loose flocculi so common in the clumping of motile bacilli are absent.

3. The agglutination appears only after a much higher degree of immunization of the animal yielding the serum than is necessary with the motile bacilli.

These observations lead to the inference that the agglutinin affecting non-motile bacilli is produced with more difficulty and acts more slowly, so far as mere optical tests go, than the agglutinins produced by motile bacilli. Furthermore, a study of the clumping process in the presence of motile bacilli with serum prepared with such bacilli indicated that the flagella were chiefly acted upon. The clumps began as loose collections of bacilli, not touching one another, but separated by narrow spaces. The entire mass floats as one, but the bonds that connect the individuals are not in sight. The clumps of the non-motile bacilli are compact. The bacilli are not separated by any appreciable space.

Those who have given attention to the clumping of motile and non-motile bacteria in the hanging drop, with immersion lenses, have without doubt noticed these differences, and so far the phenomena may be considered well known. Only by a study of the reciprocal action of immune serum upon these closely related motile and non-motile bacilli were certain new facts elicited.

Rabbits were immunized by repeated injections of living and dead cultures of the motile hog-cholera bacillus α from different sources. The serum, after four or five weeks of treatment, would as a rule show distinct clumping with a hand lens in dilutions above 1:10,000; sometimes in dilutions above 1:30,000. These figures represent limits; that is, dilutions of one-half the strength given would not show any change in the tubes after two to four hours. When the non-motile hog-cholera bacilli were exposed to the same serum, they would clump only in dilutions up to 1:500. The same was true of the serum prepared with *B. icteroides* which, as stated in the preceding paper, acted precisely like hog cholera α in all agglutination tests.

TABLE I.

Culture used for immunization.	Number of rabbit.	Agglutination towards its own bacillus.	Non-motile hog-cholera bacillus.
Icteroides (orig.)	128	8,000	500
Hog cholera, Md.	93	32,000	500
Hog cholera, Mass.	143	16,000	500
Swine dysentery α	158	2,000	< 20
Guinea-pig disease α	157	4,000	< 20

NOTE. — For the history of the cultures and the immunization of the rabbits see Journ. Med. Research, 1903, ix, p. 270.

It might be claimed that the figures above given do not establish any close relation between the motile and non-motile type of *B. cholerae suis*, but the objection is removed when we consider that the serum of none of the other more distantly related bacilli acted on the non-motile bacillus in dilutions of 1 : 20, or even 1 : 10, and that the relation given holds for hog-cholera α from various sources, as well as for its congener, *B. icteroides*.

It would seem, then, that a serum agglutinating motile bacilli acts upon non-motile bacilli of the same biological and pathogenic characters, but in dilutions at least twenty times as concentrated.

The immediate inference which might be drawn is that the agglutinins which readily act upon the flagella of bacteria are the same as those acting upon the bodies of bacteria, but they must be much more concentrated in order to produce any visible effect upon the envelope of the bacteria. If this were true, a serum prepared with the non-motile bacilli and agglutinating the latter at 1:500 should attack the flagella of the motile forms much more readily and produce agglutination in higher dilutions, *i.e.*, up to 1:10,000 and above.

But the experiment did not support any such inference. The serum produced with the non-motile bacillus acted upon

hog-cholera *a* and *B. icteroides* very much as upon the non-motile bacillus itself, as shown in table II. In general, the clumping of the motile bacillus required dilutions a trifle more concentrated, while the serum failed to act upon other bacilli of the same group in dilutions of 1:10 with two isolated exceptions (guinea-pig disease *a*).

TABLE II.

DONOR- TYPICAL ANIMAL.	Dose and number of in- jections of living cul- ture of non-motile hog- cholera bacillus.	Method of Testing.	Bacillus Coli VI.	Ty- phoid V.	Inter- trac. (Sana- relli.)	Gall- bacil- lus.	Hog cholera motile.	Hog cholera motile Janr.	Swine dysen- tery α	Sperm- ophile.	Guinea- pig disease β	Other cultures.
Guinea-pig, 2302	0.5 cc. of 48-hour bouillon culture.	Hanging drop un- der mi- croscope.	<10	10	200	<10	500	200	<10	<10	<10	{ Hog cholera, Arkansas — 500. " " Mass. — 200. " " Minnesota — 500. " " Nebraska — 500. Gall bladder — <10. ₂
Guinea-pig, 2341	0.5 cc. 48-hour bouil- lon culture. 1 cc. 24-hour culture (2 injections).	Hanging drop un- der mi- croscope.	<10	10	200	<10	200	100	10	<10	<10	{ Hog cholera, Arkansas — 200. Gall bladder — <10.
Guinea-pig, 2547	Bouillon suspension of 1 agar slant twice.	In test tubes.	—	<10	100	<10	100	50	10	<10	50	{ Bacillus Coli IX. — <10. " " X. — <10. Swine dysentery β — <20. Guinea-pig disease β — 20.
Rabbit, 139	Bouillon suspension of 1 agar slant twice.	In test tubes.	—	<10	50	<10	100	20	10	<10	20	{ Bacillus Coli IX. — <10. " " X. — <10. Swine dysentery β — <20. Guinea-pig disease β — 10.
Rabbit, 149	1 cc. 96-hour bouillon culture. 1 cc. 48- hour culture 1.5 cc. 120 hour culture (3 injections).	In test tubes.	<10	<10	100	<10	400	100	<10	<10	<10	{ Icteroides (Santiago) — 200. Swine dysentery β — <10. Shiga — <10. Hog cholera, Mass. — 100. " " Nebraska — 400. " " Arkansas — 200.
Rabbit, 153	0.75 cc. 6-day bouil- lon culture. 1 cc. 8-day culture (2 in- jections).	In test tubes.	<10	<10	200	<10	200	200	<10	<10	<10	{ Hog cholera, Arkansas — 200. " " Mass. — 200. Bacillus Coli X. — <10. " " XII. — 40.

The explanation of this behavior of the serum from the non-motile bacillus became clear on further study. Above all, the phenomenon of clumping itself was instructive. In place of the large, fluffy precipitate so frequently seen with motile bacilli, only a uniformly fine compact granular precipitate appeared, wholly similar to that in the tubes of the non-motile bacillus. Later the small clumps gathered into larger ones and subsided. The suspicion was at once aroused that the "non-motile" serum contained agglutinins for the bodies of the bacilli and not for their flagella, and that the process of agglutination affecting the bodies was qualitatively and not simply quantitatively different from that of the flagella. Further studies, especially of the clumps, failed to show anything to contradict this theory. The clumps of motile bacilli produced by "non-motile" serum do not begin with the regular spacing of bacilli, but they appear more or less compact from the start. The bacilli form compact bands which attach themselves to one another and thus form an interlacing mass when a number of smaller clumps have united. The behavior of the flagella throughout is of interest. The small clumps move about actively. Signs of paralysis are absent. When the smaller masses have drifted together, the larger clumps show throughout a protean change of outline due to the activity of the uninjured flagella.¹

In order to determine the exact relation existing between the flagellar and the body agglutinin, saturation experiments were tried to remove one or the other agglutinin. It should be borne in mind at the outset that in the non-motile serum only one agglutinin is postulated by the theory, while in the motile serum both flagellar and body agglutinins may be

¹The action of the body agglutinin upon motile bacilli is frequently seen when serum of sufficient concentration is used. In such cases, the flagellar agglutinin being present in abundance, relatively large flocculi are formed, which subside and unite into a loose, snowy mass in the bottom of the tube. This fluffy deposit, if left undisturbed, begins to shrink, and within twenty-four hours has contracted probably on account of the action of the body agglutinin into a relatively minute mass. After the clumps which form at first have subsided, a fine, powdery, or granular precipitate is frequently seen. This is probably the result of the body agglutinin acting on the bacilli deprived of flagella.

looked for, since motile bacilli stimulate the formation of both.

1. Serum¹ from a rabbit (143) treated with motile bacilli (hog cholera, Mass.) was diluted 1 to 50. Into this dilution non-motile bacilli were put from an agar slant until the fluid became quite turbid. It was placed in the incubator for four hours, centrifugalized, and filtered through filter paper, and a clear fluid was obtained. This showed that the serum had not been fully saturated, otherwise we should look for a faint cloudiness remaining. This treated serum was compared with an untreated serum in different dilutions toward the two bacilli used for immunization, also toward an old hog-cholera culture and *B. icteroides* (Sanarelli). The method employed is the same as that described in a foregoing paper, *i.e.*, small test tubes were used, and the fluid examined with the naked eye, a hand lens, and occasionally in the hanging drop, with a $\frac{1}{2}$ oil immersion objective.

The result showed a diminution of the body agglutinin toward the non-motile bacillus from 1 : 500 to 1 : 100. There was no loss of flagellar agglutinin, for the series of tubes containing treated and untreated serum presented the same appearance. The precipitate was very fluffy, and snow-like in character.

2. This experiment was repeated with serum from a rabbit treated with hog cholera, Md. The serum used in a dilution of 1 : 50 was supersaturated with the non-motile bacillus, placed in the incubator for several hours, and then in the refrigerator over night. After repeated filtration through paper a faint cloudiness still remained, showing the absence of any free body agglutinin. With this serum dilutions were made and similar dilutions of untreated serum.

The untreated "motile" serum clumped the non-motile bacillus in dilutions up to two hundred. The treated serum did not, having been supersaturated with it. Both treated and untreated serum clumped the motile bacillus in dilutions up to 1 : 20,000.

¹For details of immunization, see Journal Medical Research, 1903, Vol. ix, p. 270.

3: Serum from an immune rabbit (153) which had received two doses of living bacilli of the non-motile variety was found to have agglutinin in the following concentration:

Non-motile bacillus, 1:200 . . . trace at 1:500

Hog cholera, Md., 1:200 . . . " "

Hog cholera, Ark., 1:200.

This serum, in a dilution of 1:20, was supersaturated with motile hog cholera, Md., from agar slants, placed in the incubator for several hours and in the cold over night. Next morning the fluid was centrifugalized, but a faint cloudiness remained, showing an excess of bacilli in the presence of agglutinin.

The faintly clouded, treated serum was used for dilutions, and parallel dilutions were made with untreated serum.

In the treated serum the agglutinin toward the non-motile bacillus had fallen from 1:500 (trace) to 1:40 (trace). In the 1:40 dilution a dense granular precipitate still formed with the motile bacillus (Md.) just visible with the hand lens. This shows that even the supersaturation had left some agglutinin behind.

We may infer, therefore, that while no appreciable effect is produced upon serum prepared with the motile hog-cholera bacillus and saturated with the non-motile bacillus so far as the clumping of motile bacilli is concerned (flagellar agglutinin), the removal of the body agglutinin from the non-motile serum by saturation with motile bacilli affects the agglutinin of the non-motile bacillus, and thus indicates identity of the two body agglutinins.

Another experiment which suggested itself is the removal of the flagellar agglutinin by motile bacilli and a testing of the influence of this removal on the body agglutinin. The difficulty encountered here is the absorption of both agglutinins at the same time. The experiment was, however, made by adding to "motile serum" (hog cholera, Mass.) its own bacilli, incubating for an hour and then removing the bacilli by filtration. The filtrate was found to have lost decidedly of both body and flagellar agglutinins, in spite of the short period of incubation. The proportion of loss seems to

have been nearly the same for both motile and non-motile bacilli.

The foregoing experiments are sufficiently complete at present to warrant the conclusion that the agglutinins for the flagella and for the body of bacilli, at least so far as the large group of pathogenic colon derivatives is concerned, are distinct, not mutually interacting substances. It is obvious that but for the existence of these two varieties of bacilli (one motile and the other not) the demonstration could not have been successfully made. With the new facts in view it may now be possible to carry out more effectually Malvoz's experiments of depriving motile bacilli of cilia and demonstrating upon them the presence of a second agglutinin.

The existence of agglutinins for at least two different organs of bacilli will obviously strengthen the theory that agglutinins act directly upon the structures rather than indirectly through precipitants. The theory of Gruber-Durham that the agglutinins produce a stickiness of the parts acted upon seems on the whole the simplest explanation of the facts related. Similarly, many anomalies of agglutination depending upon partial loss of motility of cultures and upon the irregularities in the clumping of cultures of *B. coli* will be cleared up by the knowledge of at least two distinct agglutinins. The thread reaction, upon which controversies have been waged, will also be open to explanation, as in our experience it seems to be closely associated with the body agglutinin.

These experiments go a step farther in supporting the thesis presented in the preceding paper that the host is responsible, within certain limits, for the agglutinative characters of the bacillus parasitic in that host. These limits are the original capacity of the bacilli, *i.e.*, the possession of certain receptors and the degree of parasitism attained. Invasion of the blood and tissues and multiplication therein represents the highest degree, and bacteria of the same species, having acquired this power toward the same host, usually agglutinate alike. The ancestry of the motile and the non-motile races of the hog-cholera bacillus will remain in doubt,

and any speculation concerning it can do little more than stimulate efforts to elucidate it. Some years ago, in the paper referred to, one of us presented the hypothesis that the motile races are derivatives of *B. coli* or its progenitor, and the non-motile race a derivative of *B. lactis aërogenes*.

CONCLUSIONS.

1. The non-motile race and the motile races of the hog-cholera bacillus manifest a close affinity towards one another in the presence of immune agglutinins. This is also true of *B. icteroides*, Sanarelli.
2. This affinity enables us to differentiate the agglutinins of motile bacilli into flagellar and body agglutinins.
3. The agglutinin acting upon the bodies of the non-motile hog-cholera bacillus is identical with that acting upon the bodies of the motile race or species, but different from that acting upon the flagella.
4. The flagella agglutinins are much more easily demonstrated in immune sera. In the cultures studied the presence of the former was manifested in dilutions over twenty times greater than in those in which body agglutinins became visible.
5. In order to obtain body agglutinins, a much higher degree of immunity must be induced.
6. The assumption of two agglutinins as defined above will probably serve to clear up various apparent discrepancies in agglutination tests and explain the so-called thread reaction.

MUCIN AS A BACTERIAL PRODUCT.¹

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Bacteria play a very important part in the decomposition of organic matter. Whether beneficial or injurious to mankind, the transformation of substances into simpler or more complex ones, through the agency of bacteria, takes place under numerous circumstances; and an almost endless number of decomposition products may be the result. Thus, proteids are split up not only into the ordinary gastric and pancreatic digestion products, including leucin, tyrosin, and tryptophan², but into a number of other substances also; indol, skatol, aromatic oxy-acids, and mercaptan, for example. Through the action of bacteria on sugars, alcohol and a series of acids are formed, the most common being formic, acetic, butyric, valerianic, and lactic acids.

Starches may be transformed into sugars, as Eijkmann³ and others have shown; and fats are split up into their fatty acids and glycerine, as is illustrated in rancid butter. Then there are a large number of bacterial products of which comparatively little is known; of these the diphtheria and tetanus toxins are among the most important.

While bacteria are commonly regarded as breaking down more or less complex substances into their simpler components, they are also endowed with the property of building up more complex substances from simpler ones. For example, the albuminous substance of the protoplasm which they contain as living organisms must itself be a product of synthesis, especially when growth and multiplication of the organism take place in such a medium as Uschinsky's

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² Rettger. *American Journal of Physiology*, 1903, viii, p. 284.

³ Eijkman. *Centralblatt für Bakteriologie*, 1901, xxix, p. 841.

solution,¹ which is entirely devoid of proteid matter. Another illustration of bacterial synthesis is the production of mucin.

The exact chemical nature of those substances which are called mucins is still to a large extent unknown; but bodies which fall into this class are described as having certain characteristics in common. Perhaps the most important of these are: its precipitability by weak acids; its solubility in small quantities of alkalis; and its decomposition with weak acids into a proteid body and a substance capable of reducing Fehling's solution.

Mucins have been obtained from various sources, as, for example, bone and connective tissue, saliva, bile, and the slimy secretions of certain invertebrates. But the possibility of the production by bacteria of such a complex body as mucin has as yet been given very little consideration.

Weyl² claimed to have discovered a body akin to mucin among the decomposition products of the tubercle bacillus. Hunter³ substantiated this result and wrote: "This (mucin) is present in quantity sufficient to give a distinct cloudiness on adding acetic acid to strong solutions of tuberculin."

Lepierre,⁴ in his studies on a fluorescent bacillus, demonstrated that it produced mucin in "considerable quantities," especially when the organism was grown in peptone solution.

According to Lepierre, the substance has the properties of true mucin. Charrin and Desgrez⁵ observed that certain cultures of *B. pyocyaneus* had a viscous appearance. On treatment of the culture fluids with dilute mineral acids or acetic acid, a precipitate was obtained the appearance and properties of which placed it in the class of "mucinoids." This substance was produced in ordinary beef bouillon, but failed to develop in one per cent peptone solution or in

¹ Uchinsky's solution has the following composition: water, 1,000 parts; glycerine, 30-40; sodium chloride, 5-7; calcium chloride, 0.1; magnesium sulphate, 0.2-0.4, di-potassium phosphate, 0.2-0.25; ammonium lactate, 6-7; and sodium asparaginate, 3-4 parts.

² Ref.

³ Hunter. *British Medical Journal*, 1891, July 25, p. 171.

⁴ Lepierre. *Comptes rendus*, 1898, cxxvi, p. 761.

⁵ Charrin and Desgrez. *Comptes rendus*, 1898, cxxvi, p. 596.

fluids containing only mineral ingredients. On decomposition with weak acids it yielded a compound which reduced Fehling's solution.

The possibility of the occurrence of mucin as a bacterial product was suggested to the writer by Dr. E. K. Dunham, of New York, after his discovery and isolation of an organism¹ which produced very marked viscosity when grown in the ordinary culture media, especially bouillon.

The bacillus grew rapidly in beef bouillon, both at incubator and at room temperature, though the latter was more favorable. A general cloudiness was produced together with a whitish precipitate which had a pronounced tendency to stick together in slimy masses. The liquid soon became very slimy, and in the course of twenty-four to thirty-six hours the viscosity became so marked that the liquid to a large extent resembled mucus. Various culture fluids (two to four weeks old) were chemically examined. Most of these were bouillon cultures which had been incubated three to four weeks.

On treatment with dilute mineral acids or acetic acid, a precipitate was obtained which remained insoluble in excess of acetic acid and was only slightly soluble in the stronger mineral acids. The precipitate was almost white and consisted of small flakes which settled to the bottom slowly. In a few instances it had a tendency to become slightly stringy. It was readily soluble in very dilute sodium hydroxide or sodium carbonate, from which solutions the body was again precipitated on the addition of small quantities of acid.

The substance was obtained in comparatively pure form, for further examination, through repeated precipitation from its sodium carbonate solution with dilute acetic acid, followed by filtration. As a rule, five precipitations were made. The final product was thoroughly washed with water, then with alcohol and with ether, and dried.

The powder obtained in this way was grayish white in appearance. It was insoluble in weak acids, and soluble

¹ According to Dr. Dunham, this organism is identical with the so-called *Bacterium sub-viscosum* (See Migula, System der Bakterien, 1900, ii, p. 326).

in alkalis and alkali carbonates. It gave distinct biuret reaction and a test with Millon's reagent. When dissolved in very dilute sodium carbonate solution it gave a precipitate with copper sulphate and other heavy metals, as well as with strong alcohol. Neutral, or slightly alkaline solutions yielded no precipitate on boiling, but were precipitated by saturation with sodium chloride. After heating for several hours with two per cent sulphuric acid, or three and a half per cent hydrochloric acid, the neutralized decomposition mixture reduced Fehling's solution.

Elementary analysis¹ of the prepared powder gave the following:

	Per cent.	
Carbon . . .	50.3	} Including ash.
Hydrogen . . .	7.12	
Nitrogen . . .	14.37	
Phosphorus . . .	0.08	
Sulphur . . .	0.74	
Ash	2.6	

The organism under consideration grew rapidly in one to two per cent peptone solution, though the amount of mucin formed was considerably less than when growth occurred in beef bouillon or in milk. The latter was a very favorable medium, and comparatively large yields of mucin could be obtained in it. While in two per cent peptone, or even bouillon, the maximum yield of mucin was about 0.04 per cent; in fresh, skimmed milk as much as 0.1 per cent was obtained. Precipitation of the mucin from milk was accomplished by adding a large excess of acetic acid and stirring the fluid vigorously, so as to bring about complete re-solution of the casein. After five or six precipitations were made in this manner, a product was obtained which resembled in every particular the mucin obtained from bouillon cultures. That the substance prepared from milk enclosed little or no nucleo-albumin (casein) was shown by the small amount of phosphorus which it contained (0.08 per cent).

Milk remained unprecipitated by this organism until the

¹ I am indebted to Mr. P. B. Hawk, of New York, for the determination of C and H in the mucin.

fifth or sixth day (37° C.), when the casein began gradually to coagulate. The whey was only slightly acid, but was quite slimy.

The production of mucin does not depend upon the presence of carbohydrate material in the medium. Full yields of mucin were obtained in bouillon from which all traces of sugar had been removed by previous fermentation.¹ Furthermore, the addition of varying quantities of dextrose, levulose, lactose or sucrose to bouillon did not perceptibly increase the amount of mucin formed.

In pure solutions of these sugars the organism refused to develop. It likewise failed to grow in Uschinsky's fluid, and no development took place in the absence of atmospheric oxygen.

The production of mucin, or a mucin-like substance, is not limited to one or even a few species of bacteria, but is a property which many (if not all) kinds of bacteria possess, though in most cases the quantity of mucin formed is so small as to be scarcely perceptible. Culture fluids of the following organisms were examined; *Vibrio* Finkler-Prior, Müller's spirillum, *Bac. mucosus capsulatus*, *B. pyocyaneus* (two varieties), *B. fluorescens*, *B. coli commune*, *B. subtilis*, *B. megatherium*, *Proteus vulgaris*, *Micrococcus ureæ*, *Sarcina lutea*, and *Staphylococcus pyogenes aureus* and *citreus*.

Evidence of the presence of mucin was obtained in peptone or bouillon cultures of each of these organisms. However, the quantities of mucin and the ease with which it was precipitated by acetic acid varied with each particular species. Thus while the amounts of mucin produced by *B. coli commune* and *B. subtilis* were very small, their precipitation from the respective culture fluids was accomplished with comparative ease. On the other hand, culture liquids of *B. mucosus capsulatus*, which were very slimy and apparently contained a considerable quantity of mucin, were precipitated with great difficulty, and the yield of mucin was small. Comparatively large amounts of mucin were obtained from culture fluids of *V. Finkler-Prior* and Müller's spirillum, though the

¹ This was done by means of the colon bacillus. See Smith. *Journal of Experimental Medicine*, 1899, iv, p. 373.

fluids were almost free from sliminess. The mucin, in these instances, was precipitated also with little or no difficulty. Culture fluids of all the organisms became distinctly alkaline in the course of three or four weeks. This was true in particular of the organism obtained from Dunham, and of *V. Finkler-Prior*, *Müller's spirillum*, and *B. megatherium*. On treatment of the liquids with caustic soda, and distillation, an alkaline distillate was obtained which had the characteristic odor of tri-methylamine. On further examination of the distillate of the Dunham organism, it was found to contain chiefly ammonia, together with tri-methylamine, as shown by the reactions and by the melting point of the picric acid salts (crystals) of these bases.

This same organism brought about only slight decomposition, when grown in sterile egg-meat mixtures.¹ Neither indol, skatol, phenol nor mercaptan was formed. Hydrogen sulphide was present in moderate amount; and though albumoses and peptone were easily recognized, very little or no tyrosin and leucin were demonstrable. In none of the cultures was there any marked production of volatile fatty acids as shown on distillation with sulphuric acid.

The following method was employed for the precipitation and purification of mucin:

The culture fluids were filtered through a Buchner funnel, diluted with an equal volume of water, and made distinctly acid with a fifty per cent solution of acetic acid. They were then vigorously stirred and more acetic acid was added (small quantities at a time) until there was some indication of precipitation; whereupon the mixtures were allowed to stand twelve to twenty-four hours, or until the mucin was completely precipitated. In this event, the liquid which had previously been cloudy and slimy now became clear and watery. It was decanted from the precipitate, which was then washed with distilled water and collected on a filter (Buchner funnel). After repeated washing, the residue was dissolved in water containing a few drops of sodium carbonate

¹ Rettger. American Journal of Physiology, 1903, viii, p. 284.

solution. The mucin was again precipitated from its solution, filtered, washed, etc., as before. This process was repeated five or six times. After the last precipitation the decanted water was replaced by strong alcohol, and the suspension well stirred. In this way the precipitate was rendered less sticky than when the alcohol was first brought in contact with it on the filter, and the final filtration and the washing with alcohol and with ether were greatly facilitated.

The mucin organism of Dunham grew rapidly in the ordinary culture media, and from the beginning rendered them very slimy. In the course of two to three weeks (at 37° C.) much of the sliminess gradually disappeared. In peptone and bouillon cultures which were less than two weeks old, and in which the ropy consistency was still very marked, the precipitation of mucin was accomplished with considerable difficulty; and in very young cultures often became impossible. In other words, the older and the more liquid the culture fluids, the easier the precipitation.

This peculiarity in the separation of the mucin from the liquids may be due to one or both of two things. The mucin may not be in a perfect state of solution when in this slimy condition, and therefore is precipitated with difficulty by acetic acid. As the culture grows older the gradual production of alkali in the fluid increases the solubility of the mucin, and in this way the liquid is rendered less slimy.

On the other hand, there is considerable evidence that the process of mucin production by bacteria is not a direct one. There is presumably formed an intermediate body,—a “pseudomucin,”¹—to the presence of which the younger cultures owe their marked slimy consistency. As development goes on, this peculiar substance gradually becomes transformed into the more soluble type of mucin. Hence, as a rule, the older the culture the larger the amount of mucin contained in it and the easier the precipitation with acetic acid.

Two things stand out prominently in favor of the latter view. The addition of small quantities of potassium hydroxide or ammonia to culture liquids which were very

¹ Hammarsten. *Zeitschrift für physiologische Chemie*, 1882, vi, p. 194.

slimy did not materially decrease the sliminess; and in the second place, a precipitate was obtained in several instances which to a large extent redissolved on washing with dilute acetic acid or water. Such a resolution of precipitate occurred only when the latter had been obtained from a fluid of marked ropy consistency and when precipitation was accomplished with very great difficulty. Mucin obtained from older and less slimy cultures remained completely insoluble in water when once precipitated, as would be expected of true mucins. It seems probable that the precipitation of mucin from younger cultures is difficult for the reason that the fluid contains only a small amount of true mucin, and that this is held in solution by the comparatively large amount of "pseudomucin" present, which is not precipitated by acetic acid. Each body exercises a solvent or precipitating influence on the other, as the case may be. When precipitation completely fails, the "pseudomucin" holds the mucin in solution; and, on the other hand, when the mucin is perchance thrown out of solution by the acid, there is carried with it, more or less mechanically, a considerable amount of the intermediate body, which on filtering and washing goes into solution again and passes through the filter.

Attempts to isolate this intermediate body from culture fluids have remained unsuccessful thus far. With the unsatisfactory methods available it is indeed a very difficult matter to separate a substance of this nature from a solution which contains a number of other proteids.

The rapidity with which the "pseudomucin" is transformed into the final product, mucin, varies to a large extent with the bacterial species. While in cultures of the Dunham organism and of *B. mucosus capsulatus* the transformation of the intermediate body into mucin is comparatively slow, in cultures of *V. Finkler-Prior* and of *Müller's spirillum* it is presumably rapid, and in some instances appears to be extremely rapid, so that very little or no sliminess of the culture fluid becomes apparent.

(In conclusion I wish to thank Professors C. A. Herter, E. K. Dunham, and L. B. Mendel for their valuable assistance in this work.)

THE FATS OF PNEUMONIC EXUDATIONS.*

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In recent times much attention has been directed to the fats of the body, their distribution in the various tissues under normal and abnormal conditions, and the interpretation of their presence. No longer are Virchow's¹ criteria for the differentiation of fatty infiltration from fatty degeneration accepted. Rather, influenced by the work of Lebedeff,^{2, 3} Rosenfeld,⁴ Krehl and his pupils,⁵ Taylor,^{6, 7, 8} Lubarsch,⁹ Fischler,¹⁰ and others, there is a growing tendency to question even the existence of a fatty degeneration in the sense of a formation of fat in situ from the splitting up of the cell proteid and, instead, to consider that the condition is in reality a fatty infiltration with which is associated a varying degree of cell degeneration.

With this revival of interest in the tissue fats have come new technical methods and these have been applied to different tissues and processes, mainly to the liver, kidney, heart, and skeletal muscles. The purpose of this paper is to give the results of the application of some of these methods to the pneumonic lung.

The histology of pneumonia has been studied in its various aspects by Fox,¹¹ Finkler,¹² Kohn,¹³ Hauser,¹⁴ Bezzola,¹⁵ Ribbert,¹⁶ Aufrecht,¹⁷ and Pratt.¹⁸ In their papers very little or no consideration is given to the presence of fat in the various cells of the exudate. Fox¹⁹ pictures and describes small cells with rounded nuclei, a few of which, he says, show fat drops in their interior. These occur in both red and gray hepatization. Pratt,²⁰ considering desquamated epithelial cells, says: "The protoplasm stained but lightly and was frequently vacuolated. Treatment with osmic acid showed the vacuolation was not due to fat."

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In many of the text-books of pathology fatty degeneration is considered as playing an important part in the stage of resolution (Ziegler,²¹ Orth,^{22, 23} Schmaus and Ewing²⁴). Simon,²⁵ in studying the action of a proteolytic ferment in the process of resolution, notes the presence of fat in the expressed fluid after autodigestion in the incubator. However, with these exceptions, it seems that special attention has not been given to the fatty substances present in the cells of pneumonic exudations.

Technic. — For the study of the fats of the pneumonic lung several methods were employed: (a) the Marchi method for staining degenerated myelin sheaths of nerve fibres;²⁶ (b) the method of direct reduction of osmium tetraoxide ("osmic acid") by fat, the fett-osmium-reduction of Starke;²⁷ (c) the secondary reduction of fat thus treated by alcohol, the alcohol-osmium-reduction of Starke; (d) staining with Sudan III., a stain introduced by Daddi,²⁸ or with Scharlach R. (Fettponceau), recommended by Michaelis;²⁹ (e) the determination of solubility in alcohol and ether. In the first method celloidin embedding was employed, the sections cleared in chloroform and mounted in chloroform balsam; for the others frozen sections mounted in glycerin were used. All material was hardened and preserved in a ten per cent aqueous solution of commercial formaldehyde.

Sections treated with osmium tetraoxide were studied unstained, or counterstained with a saturated aqueous solution of saffranin. Those stained with Sudan III. or Scharlach R. were left unstained, or counterstained with hematein, methylene blue, toluidin blue, or methyl green in aqueous solutions. A one per cent solution of osmium tetraoxide in distilled water was made use of, and allowed to act on the sections for twelve to twenty-four hours. For staining with Sudan III. or Scharlach R., the sections were left for twenty-four to forty-eight hours in a solution in seventy per cent alcohol, supersaturated in the way suggested by Fischer³⁰ (saturate with boiling 70 per cent alcohol, and place solution

for twenty-four hours in incubator at 37° C.) or for ten to fifteen minutes in the alkaline solution of Herxheimer³¹ (absolute alcohol 70 pts., water 10 pts., 10 per cent sodium hydroxide 20 pts., saturate with Sudan III. or Scharlach R.). The former method, with an alum hematein counterstain³² gave the most satisfactory results. Of these two fat stains, Scharlach R. was found preferable, because it gives a rather brighter staining of the droplets of fatty substance, does not tint other tissue elements of the lung, and has less tendency to precipitate in the preparations.

The results obtained by these methods were controlled by paraffin sections stained with hematein and eosin. Unless this is done, error may arise with the osmium preparations from mistaking for fat, pigment already present in the lung either hematogenous or extraneous in origin. Further, in material preserved in formaldehyde a dark, granular precipitate sometimes appears. All of these substances may present much the same appearance as osmium reduced by fat and lead to confusion. Another source of error as pointed out by Poll³³ may arise from the reduction of the stain subsequently used by remains of osmium tetroxide in sections not thoroughly washed in water.

Material.—Typical examples of the various forms of exudation in pneumonia have been studied, representing the earlier and later stages of the disease. Tissue from different portions of ten lungs has been examined. The classification of the cellular exudate given by Pratt¹⁸ has been followed.

In one lung the exudate consists of non-granular mononuclear cells, with a smaller number of polymorphonuclear leucocytes, a varying admixture of fibrin, and a few scattered, desquamated, epithelial cells. The cells, with very few exceptions, are well preserved, and show no evidence of degenerative changes. In the polymorphonuclear leucocytes of both alveoli and bronchi are numerous droplets of a substance which stains bright orange-red with Sudan III. and Scharlach R. These droplets are small. As a rule, they measure less than one micron in diameter, though occasionally

they are larger. The number in a single cell varies from one to twenty or more; generally there are six to ten. They are irregularly distributed in the cytoplasm, sometimes grouped together, but do not occur in the nucleus. In many alveoli they are found in almost every polymorphonuclear leucocyte. They are sometimes found in the mononuclear cells, but oftener these are free from them. They appear also in some of the desquamated epithelial cells. Besides these small droplets, a cell is occasionally seen which contains droplets two or three times their size, and staining similarly. All of these cells lie within the alveolus or bronchus. Sometimes an intravascular leucocyte contains similar small droplets.

Sections from this same lung treated by any of the osmium tetroxide methods give very different pictures from the above. In them the mononuclear and polymorphonuclear cells very infrequently contain droplets of a brown or black color. The very few droplets present are larger in size than the ones common in the Sudan III. or Scharlach R. preparations. As a rule they are not very dark in color, though they may become darker after secondary reduction in alcohol. In the desquamated epithelial cells are numerous masses, rather coarse, often irregular in contour, and black or brownish-black in color. Examination of paraffin sections, untreated with osmium tetroxide, shows that most of the latter and some of the former are pigmented masses already present and not reduction products of the osmium treatment. That the droplets staining with Sudan III. or Scharlach R. are present and unaffected by the osmium tetroxide is shown by their appearance in large numbers when osmium preparations, not previously treated with ether or strong alcohol, are stained with one of these fat stains.

All of these droplets before osmium treatment are soluble in ether and less readily soluble in strong alcohol, as shown by their not appearing in section stained with Sudan III. or Scharlach R. after being extracted with ether or strong alcohol. Eighty per cent alcohol does not dissolve them, or dissolves them extremely slowly.

In another lung the exudate contains a larger number of polymorphonuclear leucocytes and numerous phagocytic cells with leucocyte inclusions. In this are found many more droplets which reduce osmium tetroxide than in the first. The number of these, however, is greatly exceeded by the droplets staining with Sudan III. or Scharlach R., and this increment appears to come largely in the form of small droplets.

In this lung the phagocytic cells are of interest. The included cells are almost always polymorphonuclear leucocytes or their remains. Some of these included leucocytes contain numerous orange-red droplets, while the cytoplasm of the phagocyte is free from them. In others both phagocyte and inclusion contain these droplets or they may be present only in the cytoplasm of the phagocyte, while the ingested leucocyte shows more or less evidence of digestion, or they are absent from both. Those inclusions in which the digestive process is advanced practically never contain fat droplets. Also there are present large cells, containing no inclusions, but otherwise similar to the phagocytic cells, which are either free from or contain orange-red droplets. Many though not all of these droplets also reduce osmium tetroxide.

These appearances suggest that the leucocyte is ingested with its fatty content, and that in the process of intracellular digestion the fat droplets are removed from the leucocyte and reappear in the phagocyte. The leucocyte generally lies in a distinct vacuole. There is no evidence of a direct passage of the droplets through this, and it seems probable that here we have to do with a process similar to that in absorption of fat from the intestine, where, from the work of Kischensky,³⁴ it appears that most of the fat passes through the epithelial cells in some soluble form, during which condition it is not stainable by any of the fat staining methods, though, after its passage, it reappears in the form of stainable droplets. This presupposes a fat splitting with subsequent fat synthesis due probably to an enzyme action. The present view of the reversibility of the action of enzymes

as advanced by Hill³⁵ and by Kastle and Loevenhart³⁶ and the regular occurrence of the fat splitting enzyme, lipase, in the blood serum and many organs, as shown by Hanriot³⁷ and Loevenhart,³⁸ give further support to the view that the fatty substance present in the leucocyte is split up, passes into the cytoplasm of the phagocyte, and is there built up again into its original form.

In lungs representing the latter stages of gray hepatization or beginning resolution, in which the cut surface of the lung has lost its granular appearance, exudes much purulent fluid, and begins to have a yellowish tinge, microscopic examination shows the remaining fibrin to be granular or coalesced into irregular masses, and the cells of the exudate, mainly polymorphonuclear leucocytes, to be very granular and vacuolated. The cells are evidently undergoing rapid degeneration, and are being absorbed into the lymphatics. These cells are filled with droplets, which reduce osmium tetroxide and which stain with Sudan III. or Scharlach R. The droplets are generally rather large, and the only difference between the two kinds of preparations is a possible greater richness of droplets in those stained with Sudan III. or Scharlach R. The lymphatics are crowded with the same cells containing the same droplets.

In one lung with a hemorrhagic exudation into the alveoli there are numerous vacuolated, desquamated, epithelial cells, but few other nucleated cells. This is from an autopsy in which the consolidation occupied the central portion of several lobes in a patient who had chronic diffuse nephritis, dilatation and hypertrophy of the heart, and general anasarca. Here the epithelial cells, both desquamated and attached to the alveolar wall, are crowded with rather large droplets staining intensely with Sudan III. and Scharlach R. and reducing osmium tetroxide actively.

Similar cells are very numerous in a case of unresolved pneumonia in which organization of the exudate is advanced and which is complicated by a disseminated tuberculosis.

Discussion. — In the earlier stages of pneumonic exudation

there is present in the cells, particularly in the polymorphonuclear leucocytes, a substance in the form of small droplets, which is soluble in strong alcohol and in ether, insoluble in water and in 80 per cent alcohol, which stains intensely with Sudan III. and Scharlach R., and which does not reduce osmium tetroxide. In the later stages, in addition, there is present likewise in the cells, generally in the form of larger droplets, a substance which has the same solubility properties, which also stains intensely with Sudan III. and Scharlach R., but which does not reduce osmium tetroxide. In other words, the exudation cells of pneumonia contain two substances of a fatty nature, differing in some of their microchemical reactions, the one appearing earlier than the other.

Two questions naturally arise: What are these substances? What is their source?

As to their nature two hypotheses may be advanced: (*a*) the two substances are the same in chemical composition, but differing in physical condition; (*b*) they are chemically different substances. According to the first, it may be supposed that one is in a more finely divided state than the other, or possesses some form of envelope, possibly albuminous in nature, to keep it in a state of emulsion, and that under these conditions it is not capable of being penetrated by osmium tetroxide, and so there is no reduction. Against this hypothesis is the following: Elsewhere, as in the heart and kidney under conditions regarded as fatty degeneration, equally fine droplets appear which do reduce osmium tetroxide. In milk where there is fat in a state of emulsion with a supposed albuminous envelope there are a few very fine droplets. The staining reactions of these can be studied in this way. Agar-agar such as is used in nutrient media for the growth of bacteria can be freed from fat by means of ether, melted, and before it cools mixed with a small quantity of milk. After solidifying, frozen sections of this mixture can be cut and treated in the same way as the frozen sections of lung. Doing this no evident difference in the richness of small droplets is found whether treated with osmium tetroxide or stained with Sudan III. or Scharlach R.

The second hypothesis, that we have to do with two substances chemically different, appears more plausible. That substances with differing staining affinities are chemically different is generally accepted, though the converse is not true. Altmann³⁹ has shown that osmium tetroxide is reduced by olein and oleic acid. As he puts it: "Das Osmium ist mithin nicht ein Reagens auf Fette in Allgemeinen sondern auf freie Oelsäure und Olein;" and on the presence of these depends the reaction of body fat with osmium tetroxide. The work of Starke²⁷ and Handwerck⁴⁰ go to show that the palmitin and stearin components of body fat do not reduce osmium tetroxide directly. Michaelis,⁴¹ Fischer,⁴⁰ and others state that Sudan III. and Scharlach R. stain all fats and these alone. Kaiserling and Orgler⁴² have studied in the adrenal, thymus, and elsewhere a substance which resembles ordinary fat, but which differs from it in being doubly refractile to polarized light and in staining only light gray with osmium, though staining red with Sudan III. and Scharlach R. These fatty substances evidently differ in staining reactions, as do those found in pneumonic exudations.

Of the two fatty substances in the lungs, the one occurring later in the disease agrees in its staining properties with body fat and is probably true fat. The one occurring early differs from true fat. It may be some form of palmitin or stearin; it may be the same as the substance described by Kaiserling and Orgler⁴² and spoken of by them as myelin; or it may be some other fatty substance. The exact nature of it cannot be determined by its staining reactions. That it is not true myelin, such as occurs around nerve fibres, is shown by the fact that it does not reduce osmium tetroxide. That true myelin is not present in our preparations appears from the fact that there is no difference between sections treated directly with osmium tetroxide and those treated by the Marchi method. The chemical analysis of Orgler⁴³ also show differences between the substance found by him in the thymus and the myelin of the central nervous system.

What is the source of the two substances? The one is present early and is sometimes noted in the leucocytes while yet intra-vascular. This suggests that the substance is present in the leucocytes before they leave the blood vessel, and so forms a passive part of the exudation process. This is supported by the above observation and by finding the same droplets in leucocytes obtained from the circulating blood of pneumonia patients. Smears were made in the usual way of blood obtained by ear puncture.* These were placed in 10 per cent formaldehyde and then treated in the same way as the frozen sections of lung. The blood from nine patients was thus examined. The leucocytes of all showed the presence of droplets in every way identical with those found in the lung alveoli. Though it may be true that not all of this substance reaches the lung within leucocytes, a considerable part certainly has this source.

Does the other substance reach the lung alveoli in the same way? From the observations of Arnold⁴⁴ and others, we know that fat is often transported by leucocytes, and undoubtedly some in the lung may come in this way. However, as the true fat appears mainly late in the disease, when the migration of leucocytes is no longer actively taking place, it seems probable that the greater part is taken up by the cells in the exudate from the surrounding fluids in which it is present, and owing to injurious conditions acting on the cells is not utilized, but stored as fat — a fatty degeneration in the sense at present accepted.

Conclusions. 1. In pneumonic exudations two kinds of intracellular fatty substances are present. 2. The one differs in some reactions from ordinary body fat, appears early in the disease, and is in the main brought by the leucocyte from the circulating blood. 3. The other is identical in reactions with ordinary body fat, appears late in the disease, and has the same origin as the fat of so-called fatty degeneration elsewhere.

* These smears were obtained with the assistance of Dr. N. K. Wood, medical house officer at the B. C. H., and to him and to the medical service my thanks are due.

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OBSERVATIONS ON THE COAGULATION TIME OF THE BLOOD AND THE BLOOD PLATES.¹

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About the middle of the last century variations in the coagulation of the blood were deemed of great importance in the study of disease. The amount of fibrin formed in shed blood was considered an index of the patient's condition.

Since that period anomalies of the coagulation of the blood have been regarded as of little significance. This has been in part due to the recognition of the fact that the methods used by the early investigators were unreliable in more than one respect. The methods for determining the coagulation time were particularly faulty. The statements in regard to the length of time which elapsed from the beginning to the completion of coagulation showed extraordinary variations.

In 1878 H. Vierordt² attempted to determine the coagulation time with greater exactness than had hitherto been done, and since then other methods have been introduced by A. Wright³ and by Brodie and Russell.⁴

The method of Brodie and Russell is the most satisfactory. A current of air is directed by means of a pointed tube, connected with a rubber bulb, against a drop of blood placed in a moist chamber. The corpuscles are set in motion. The drop is examined under the microscope. When coagulation begins movement ceases.

This was the method I adopted in an investigation which I undertook at the suggestion of Professor Krehl to determine the variations of the coagulation time in health

¹ Read May 14, 1903, at the Third Annual Meeting of the American Association of Pathologists and Bacteriologists, Washington, D.C. Received for publication, June 1, 1903.

² Vierordt, H. *Archiv der Heilkunde*, 1878, xix, p. 193.

³ Wright, A. *British Medical Journal*, 1893, ii, p. 223.

⁴ Brodie and Russell. *Journal of Physiology*, 1897, xxi, p. 403.

and disease and to inquire into the causes of these variations.¹

If several drops of blood were taken in rapid succession from the same wound, and the coagulation time estimated with different instruments, the results always agreed. This proved that the method was reliable for comparing variations in coagulation time. It was then sought to determine whether the same blood, under the same external conditions, examined with the same apparatus, always showed the same coagulation time. This experiment is difficult to perform, because the blood after it has left the vessels undergoes rapid and variable changes. These changes appear to be the result of contact of the blood with the tissues, and in the ordinary process of blood coagulation in man this contact always occurs. It was found that blood obtained by puncturing the skin of the ears or fingers of the same individual showed remarkable variations in the coagulation time.

If a large, deep cut were made from which the blood flowed freely, the coagulation time was relatively slow, while blood from a small superficial cut coagulated quickly. For example, in one case blood from a superficial cut of the skin coagulated in two minutes, while blood from a deep cut in the same individual coagulated in seven minutes.

If blood is forced out of a wound of the skin by pressure on the tissues the coagulation is accelerated.

A second drop of blood obtained from a cut after the first drop has coagulated always coagulates more rapidly than the first. This occurs also if the skin after the removal of the first drop is cleaned carefully with water, alcohol, and ether.

An increased amount of lymph in the tissues does not accelerate the coagulation time, for it was found that blood from very edematous tissues did not coagulate more rapidly than blood from non-edematous parts of the body.

¹ With the kind assistance of Professor Grützner, a modification of the Brodie and Russell apparatus was devised. This was made for us by Alberch, of Tübingen. The original instrument was obtained from London. Our modification was considerably cheaper than the original model. The price of the London coagulometer was \$10.50, while the Grützner model cost seventy-five cents, and was just as trustworthy.

Blood from very deep wounds of the skin shows no essential difference in the coagulation time from that of blood from wounds of ordinary depth. The average coagulation time of such blood determined at room-temperature is four to five minutes.

There were no characteristic differences in the coagulation time dependent upon the part examined. Blood from different parts of the body of the same individual, examined at the same time, showed striking differences in the coagulation time.

For the repeated examination of the same blood it was necessary to avoid contact of the blood with the tissues, and this was accomplished by inserting a hollow needle into the veins of the arm. Blood was obtained from sheep by puncturing the jugular vein, and from anesthetized rabbits by passing a needle through an intercostal space into the left ventricle after having first dissected away the muscles from the left front of the thorax. Three coagulometers were used for each determination. (Two of the Grützner pattern and one English instrument.) The results always agreed.

COAGULATION TIME OF THE BLOOD.

Sheep	Jugular vein	6'	45"	6'	6'	30"	6'	15"
Rabbit	Left ventricle	7		7	8		7	15
Man	Vein of arm	6		7				

The coagulation time can be determined with a fair degree of accuracy by this method, but as it involves the withdrawal of the blood directly from the veins it was not considered feasible.

The attempt was then made to discover the factors which caused the variations in the coagulation of blood obtained from puncture of the skin.

Numerous determinations of the coagulation time in health and in various morbid conditions failed to yield any positive results.

In disease as in health, the average coagulation time was from four to five minutes, and the results obtained from the

examination of the sick were in accord with the observations made upon healthy individuals. Extraordinary variations in the coagulation time occurred — from two to nine and a half minutes within half an hour in one case. No explanation of this could be given.

The relation which blood-plates might bear to coagulation was next considered.

It was necessary in the first place to devise a method of counting the blood-plates, in order to answer the question whether variations in the coagulation time depended upon variations in the number of plates.

The reason such widely different statements in regard to the normal number of blood-plates have been made is certainly in part due to errors in the methods of counting, but chiefly to imperfect methods of preserving them.¹ In the employment of any method it must be remembered that the plates outside the vessels are very vulnerable.

Of great importance was the discovery of Deetjen² that solutions of sodium metaphosphate preserve the plates.

The attempt to count the plates in a Zeiss chamber by the Thoma method was not satisfactory, owing to technical difficulties which possibly may be removed in the future.

We used a somewhat different procedure. The fresh blood was mixed with a five per cent solution of crystallized sodium metaphosphate in the proportions of one part of blood to five or ten of the preservative and a drop of the mixture placed on a carefully cleaned slide and covered with a cover-slip. The ratio of blood-plates to erythrocytes was determined and the number of plates per cubic millimeter calculated from the number of erythrocytes present. An Ehrlich ocular was used and a Leitz one-twelfth oil-immersion lens. The erythrocytes preserve their normal appearance. The plates can be distinguished without difficulty. It is of importance that all glass which comes in contact with

¹ Compare Brodie and Russell, *Journal of Physiology*, 1897, xxi, 390. Van Emden, *Fortschritte der Medicin*, 1896, xvi, pp. 241, 281. Determann, *Deutsches Archiv für klin. Medicin*, 1898, lxi, p. 365.

² Deetjen. *Virchow's Archiv*, 1901, clxiv, p. 239.

plates should be cleaned with the greatest care. We have followed Dekhuyzen's method.¹ The solution of sodium metaphosphate should be freshly prepared. Usually fifty fields in each of two drops were counted. The method is not perfect, but it appeared to be more reliable than any other.

The difficulty of obtaining an accurate count is in part due to the marked tendency of the plates to adhere to foreign bodies. They attach themselves to the glass used in making the preparation unless it be perfectly cleaned. Bizzozero² and more recently Dekhuyzen have called special attention to this property of the blood-plates. They also stick to one another and may form large clumps about the foreign body. Thus the distribution of the plates in different parts of the preparation may become very unequal. Properly prepared specimens with sodium metaphosphate solution show no clumping.

It can readily be seen that the sources of error in the examination of plates are considerable. When strict attention was paid to all the necessary precautions the number of plates in some individuals was found to be quite constant.

For example: S. Male, aged twenty-five years, laborer. Neurasthenia.

	July 2,	3,	5,	6,	7,
Erythrocytes..	4,800,000	4,960,000	4,448,000	4,160,000	4,480,000
Plates.....	400,000	496,000	448,000	346,000	407,000

L. Male, aged twenty-two years, medical student. Psoriasis.

	July 10,	11,	12,
Erythrocytes	4,960,000	4,960,000	5,200,000
Plates.....	276,000	236,000	217,000

In other cases there were notable variations in the count from day to day and hour to hour. The ingestion of food has no constant influence on the number of plates. Upon this point a number of observations were made which will not

¹ Dekhuyzen. *Anatomischer Anzeiger*, 1901, xix, p. 529.

² Bizzozero. *Virchow's Archiv*, 1882, xc, p. 261.

be given in detail here. The number of plates in the blood of the same individual at the same hour on different days sometimes shows marked differences. In one case as high a ratio as one plate to thirteen erythrocytes was noted one day and as low as one to thirty-two on the following day at the same hour.

The number of plates was estimated by this method in a large number of healthy and sick individuals. The results were no more constant or intelligible than those obtained in the examination of the coagulation time of the blood.

Only a few definite statements can be made. The plates always disappear so soon as coagulation begins. If a drop of blood is allowed to remain upon the skin, there is either a very slight diminution or none at all until the onset of coagulation. Then they vanish.

In defibrinated blood only an occasional plate is seen.

If albumose is injected into the circulation of a dog, the plates within a few minutes are found to have entirely disappeared from the blood. At the same time, as is known, the blood loses its power of coagulation.

But the failure of the blood to clot is due only indirectly, if at all, to the absence of blood-plates. For if albumose be injected a second time into a dog, the coagulation of the blood is not inhibited, but the plates disappear as before.

A few hours after the first albumose injection blood withdrawn is still completely uncoagulable, but the plates have returned in almost normal number.

In blood rendered uncoagulable by the injection of histon only an occasional plate was found.

There is no direct relation between the time of coagulation and the number of plates present in the blood. This is not only shown by experiments on animals with albumose, but we have demonstrated it repeatedly by observations on man. For example, the coagulation time of the blood obtained from a very superficial wound of the skin was two minutes, and the plate count was 172,000 per cubic millimeter. Blood from a deeper cut made in the same individual at the same site coagulated in five minutes, and contained 223,000 plates

per cubic millimeter. A second drop of blood out of the second cut showed a coagulation time of three and one-half minutes. The number of plates was 250,000 per cubic millimeter.

These observations make the view untenable that the coagulation time is determined by the number of plates in the unit volume of blood or is due to the presence of a certain amount of coagulation-producing substance derived from the plates.

We have not taken up the interesting problem of the relation of the plates to the erythrocytes and leucocytes, because it had no direct bearing upon the question we have attempted to answer, and for this reason the extensive literature dealing with this subject will not be considered. Who, however, discusses the origin of the plates must recognize the fact that in the solutions of sodium metaphosphate the extrusion or "Abschnürung" of portions of the erythrocytes was rarely seen. These products of the injured or degenerated erythrocytes present an entirely different appearance from the plates.

In our preparations of blood diluted with sodium metaphosphate solution the number of plates remained unchanged for days, as repeated counts proved.

When blood was mixed with a little hypertonic magnesium sulphate solution, Arnold's bodies were found in great number, but only a few plates were present.

THE PHYSICAL CHEMISTRY OF MILK.¹

(PRELIMINARY NOTICE.)

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Many observations in Physical Chemistry, and more recently in Physiology, emphasize the greater importance of the number of molecules in a solution, that is, of the molecular concentration, than of the size of the molecules, or the "total solids." This conception, with its associated theory (van't Hoff's theory of solution), has, indeed, made clear another form of physiological work — the work of concentration in the renal secretion.

In general, according to this theory, it is true that, if a gland secretes a fluid of higher molecular concentration (osmotic pressure) than the blood which it receives, thereby it does work; and it does not perform this kind of work, at least, if its secretion is of the same osmotic pressure or of a lower one than the blood which it receives.

The subject of this investigation is the molecular concentration of cow's milk.

The problem, not very different from the one which Bugarszky and Tangl² encountered in the case of blood serum, is first, to determine the total molecular concentration of milk (C), the concentration of electrolytes, both ions and undissociated molecules (C_E), and of non-electrolytes (C_{NE}) (of course, $C = C_E + C_{NE}$); then, so far as possible, to assign to lactose, the phosphates, chlorides, salts of calcium and potassium, etc., their part in the concentration.

Fat is not to be considered, for it is not in solution. Casein, also, does not enter into the question, for its condition is not ordinary solution, inasmuch as it always exists in milk as an opalescent solution which cannot pass through a Chamberland

¹ Received for publication May 7, 1903.

² Bugarszky and Tangl, *Pflüger's Archiv.*, lxxii, 531.

filter. The remaining proteids have very little effect on account of their high molecular weight.¹

The molecular concentration of milk, that is to say, the total number of gram-molecules of every kind which it contains in solution, may be rapidly and accurately determined by the freezing-point method, provided, as I have found, the fat is first removed by centrifugalization. If this precaution is not taken, no sharp reading of the thermometer can be made, perhaps because ice collects slowly on the surface of the fat globules, thus preventing the sudden freezing with the attendant sharp rise of the thermometer, on which the accuracy of the method depends.

Using this method, I have obtained the following results :

Δ = Depression of freezing point.

C = Corresponding molecular concentration calculated therefrom.

Δ	C.
0.540°	0.286 n.
0.586	0.310
0.560	0.296
0.557	0.294
0.590	0.312
0.570	0.301
0.540	0.286
—	—
Average, 0.563°	0.298 n.

Accordingly, as the third figure is of no value, $C = 0.30$ n.²

Bugarszky and Tangl³ found for the blood serum of the cow

	Δ	C.
the following average values	0.611°	0.323 n.

With one exception, all the results of these observers with

¹ If we assume the low estimate 10,000 for the average molecular weight of the proteids of milk, their solution is only 0.0035 n. or, excluding casein, 0.0006 n.

² These results, and all others in this paper unless otherwise stated, are from mixed milk taken from seventy-two cows of four different breeds.

³ Loc. cit.

cow's blood serum are higher than all of mine with milk. The difference, however, is slight :

$$0.611^{\circ} - 0.563^{\circ} = 0.048^{\circ} \quad 0.323 \text{ n.} - 0.298 \text{ n.} = 0.025 \text{ n.}$$

This comparison proves that in the total the mammary gland of the cow does no physical work of solution concentration, but does utilize nearly all the head of osmotic pressure of the blood plasma, by no means, however, simply to dialyze its own constituents.

Contrasted with the molecular concentration, the total solids have a special value, for they permit the determination of the average molecular weight of dissolved substances, and accordingly throw light on the problem of the relative number of molecules (and ions) of complicated and simple substances. The total solids, determined by the routine method of technical analysis of milk, and expressed in grams per liter, were as follows :

95.6
95.3
96.0
98.3
98.2
98.5
Average, 97.0

From this value and that of C., the average molecular weight is thus calculated :

$$\begin{aligned} \text{Average molecular weight} &= \text{Total Solids} \times \frac{1}{C} \\ &= 97 \times \frac{1}{0.30} \\ &= 323 \end{aligned}$$

At least eighty-five of the ninety-seven grams of solids per liter consist of lactose whose molecular weight is 342, and proteids whose molecular weight is many times greater; there is, too, a large amount of calcium phosphate which has

a high molecular weight. Accordingly, it is immediately apparent that, even in milk, salts of low molecular weight, with their ions, must constitute a considerable per cent of all dissolved molecules.

The determination of the quantity of electrolytes in milk rests first upon its electrical conductivity. For this I used the ordinary Kohlrausch method, and obtained the following values for the specific conductivity :

$$\begin{array}{r}
 \lambda_{18^{\circ}} \\
 4.44 \times 10^{-3} \\
 4.45 \times 10^{-3} \\
 4.45 \times 10^{-3} \\
 4.47 \times 10^{-3} \\
 \hline
 \text{Average, } 4.45 \times 10^{-3}
 \end{array}$$

This value, corrected for proteids, which, according to Bugarszky and Tangl,¹ lower the conductivity about 0.25 per cent per gram per liter, yields: $\lambda_{18^{\circ}} = 4.85 \times 10^{-3}$

The chlorine content, determined by Volkard's method, was, in the average, 0.95 grams per liter. This chlorine is almost entirely held by the univalent radicals, sodium and potassium, which are dissociated to the same degree, so that it is an accurate assumption to count the effect of the chlorine on the conductivity that of the equivalent amount of sodium chloride. This would be 2.66×10^{-3} , leaving $4.85 \times 10^{-3} - 2.66 \times 10^{-3} = 2.19 \times 10^{-3}$ to be assigned to salts of bivalent and trivalent acids. It follows from the smallness of this value that the phosphates must be little ionized and may well be combined.

The above determinations of the conductivity of milk yielded such constant values that I determined the conductivity of a number of samples taken at random from shops in Boston. The results were as follows :

¹ Loc. cit.

λ 18°	λ 18°
4.19×10^{-3}	4.42
4.27×10^{-3}	4.17
4.28×10^{-3}	4.44
4.01	3.99
4.10	4.01
4.42	4.34
4.06	4.08
3.90	3.92
4.54	4.17
4.12	3.94
3.92	3.99
4.12	4.23
4.34	4.19
—	—
Average,	4.16×10^{-3}

These figures are lower than those given above, because the fat was not removed.

The range of variation is here narrow enough to point to the possibility of basing a quick method for testing the concentration of milk upon its conductivity.

MERCURIAL DIURESIS.¹

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Mercury was first used externally as an antiparasitic by the ancient Greeks, Romans, and Arabs. The latter used it especially to combat scabies.

The ancients avoided using mercury or other metals internally because they feared poisonous effects. It was Paracelsus, in the sixteenth century, who introduced mercury as a specific against syphilis, which was then raging through Europe. He used it internally, by inunctions, and by fumigations.

In 1760 Casper, in his inaugural dissertation upon calomel, described it as a general panacea. It was at this time that a number of physicians noticed an increased diuresis after the use of mercury in patients suffering from heart diseases. For this reason it was used as a diuretic in practically all conditions where increased urinary flow was desired. The results were not as satisfactory as was first anticipated, so the use of calomel as a diuretic was discontinued until 1886. In this year Jendrassik again proposed the use of calomel as a diuretic, especially in cases of dropsy. This author thought there was some relation between the diuresis and the dropsy.

Binz, in 1891, recommended mercury as a diuretic only in cases of heart disease, and said that no diuretic effect is noticed when administered in cases of pleural exudates, nephritic or liver dropsies. He nevertheless believed that it produces a slight diuresis in normal individuals, and explained mercurial diuresis by stimulation of the renal epithelium.

Locke tried to explain mercurial diuresis by the increased excretion of urea, which follows the increase in metabolism. Cohnstein, in von Schroeder's laboratory, tested the diuretic

¹ Received for publication May 22, 1903.

effect of mercury and sodium hyposulphite. He observed a great diuresis when the animals were anesthetized with urethane and morphine, but no diuresis at all when chloral was used as a narcotic. He concluded from these observations that the diuresis is due, at least in part, to changes in the circulation, and that it does not occur after the use of chloral, because the latter paralyzes the peripheral circulation. He also believed that this circulatory effect is under the influence of the nervous system, because he was unable to obtain a diuresis after section of the renal nerves. This author had not excluded the action of sodium hyposulphite itself upon the urinary secretion. In view of the fact that the presence of a diuretic action from mercury had not yet been absolutely established, it seemed to us that this subject would make a fruitful line of investigation.

After having performed a preliminary series of about fifty experiments upon rabbits, cats, and dogs with different preparations of mercury we have been led to disagree with the conclusions of Cohnstein. In order to exclude the action of sodium hyposulphite, which we have found at one time to produce a diuresis of several hundred per cent, we used preparations of mercury combined with casein and albumin. After we had determined that casein had no influence upon the secretion of urine, we then studied the influence of the caseinate of mercury.

After intravenous injections of mercury we failed to obtain a true diuretic effect. The slight increased secretion which sometimes occurred was always very transient, and after subsiding it would never be increased again by further injection of the drug. Throughout all these experiments there were evidences of more or less severe renal irritation as shown by the presence of blood and casts and by diuresis.

Unlike Cohnstein we found no difference in our results whether the animal was anesthetized with chloral or with urethane. After subcutaneous administration we obtained a diuresis in a few instances. It is evident from these results that at least a large part of the action of mercury on the kidney is a purely irritant one like that of cantharides, and

that a nervous and circulatory action can hardly come into play. The fact that a diuresis was obtained a few times after subcutaneous administration but strengthens this view, because under such circumstances the absorption is slow and the quantity which will just irritate enough to produce an increase of secretion and not a decrease can be more easily reached. The decrease in secretion or anuresis, which occurred later, are just such symptoms as could be expected from irritation of the kidney.

Even though we could not obtain a constant diuresis in cats, dogs, and rabbits, after the administration of mercurial preparations, it would have been unjustifiable to conclude that under no conditions could a diuresis be produced. Since mercury is used clinically as a diuretic in cases attended with dropsy it occurred to us that it might be instructive to study the action of mercury upon animals in which artificial dropsy had been produced. We first experimented to see whether an artificial dropsy would in itself produce a diuresis, and we found that if the peritoneal cavity is kept filled with an isotonic salt solution practically no diuresis occurs in dogs and cats, but a very significant one occurs in rabbits. Dogs with artificial dropsy treated with mercury caseinate showed little or no diuresis. One cat showed quite a marked diuresis. Our experiments with rabbits were performed with an abdominal infusion consisting of isotonic salt solution containing gum acacia enough to render it colloidal, in order that absorption might not take place too readily. Such ascites alone did not produce diuresis. When, with this ascites, mercury caseinate was administered, two-fifths of the rabbits showed a diuresis of five to seven hundred per cent.

The irregularity of results obtained explain, to a great extent, the discrepancy between the opinion of different clinicians as to the value of mercury as a diuretic. There is little doubt but that individual difference in susceptibility toward mercury is very great and that the diuretic action is complicated by two factors, — local irritant action upon the kidney, and possibly an action upon serous membranes influencing the absorption of fluids from them.

ON A DIFFERENCE IN THE INFLUENCE UPON INFLAMMATION
BETWEEN THE SECTION OF THE SYMPATHETIC NERVE
AND THE REMOVAL OF THE SYMPATHETIC GANGLION.¹

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Ever since it was discovered that section of the sympathetic is followed by increased temperature and redness of the corresponding ear in rabbits, the influence of the section of the sympathetic nerve upon the course of an inflammation artificially produced in the rabbit's ear has been the subject of repeated investigations. The influence of the section of the auricular nerves has been also a frequent subject of study. The effect of these nerves, however, was considered to be chiefly a sensory one. In a recent investigation of the vasomotor nerves of the rabbit's ear, we³ arrived, however, at the conclusion that in a majority of cases the vasomotor action of the third cervical nerve on the rabbit's ear was much greater than that of the sympathetic nerve. On the basis of these results we started to subject to a new revision the old data regarding the influences of the section of the sympathetic and the cervical nerves upon inflammation. Our studies were confined to the observation of the influence of the section of the nerves upon the onset, appearance, and course of a local inflammation in the ear.

Our experiments have demonstrated to us repeatedly that, though the section of either of the nerves has in the great majority of cases a more or less distinct aggravating influence upon the inflammation, this influence is by no means in proportion to the vasodilatation produced by the section of these nerves. We had animals in which section of the third cervical nerve brought on a considerable vasodilatation in the corresponding ear. Nevertheless, the inflammatory process in this ear was far behind that produced in the ear,

¹ Received for publication, June 8, 1903.

² Research Scholar of the Rockefeller Institute.

³ S. J. and Clara Meltzer. *The American Journal of Physiology*, ix, 1903, p. 147.

the sympathetic nerve of which was cut, and the consecutive vasodilatation was very moderate in comparison. However, it is not this experience which we wish to speak about at this time. We mention it incidentally as we may have to refer to it again later on. The observation which we wish to report here deals with a difference in the influence upon inflammation between the section of the sympathetic nerve and the removal of the superior cervical ganglion.

As an introductory statement we may say that in all our experiments the inflammation in the ear was distinctly aggravated by the preceding operative interference of either kind, whether section of the sympathetic or removal of the ganglion. By aggravation of inflammation we mean that the redness set in first, that hyperemia and edema were more intense and spread over a larger area, that finally the pustules developed earlier and were larger on the operated side than on the other, unoperated side, or in the ears of an unoperated control animal. We have produced inflammations by injecting subcutaneously in the convex side of either ear an equal quantity of an eighteen to thirty hours bouillon culture of staphylococcus pyogenes aureus, or by injections of a drop of spirit of turpentine. In the latter case, care should be taken that the injected quantity is not too large. The same applies to the virulence of the staphylococci which should be not too great, as in either case the inflammation in both ears would become so intense as to make it quite difficult to recognize small differences between the two inflammatory foci. We have lately, however, employed a method which brings out the differences between the two foci very clearly even in extensive inflammations of the ears. The method consists in subcutaneous injections of a sufficient dose of adrenalin. The effect is very interesting and instructive. The difference between the inflammatory focus and the inflammatory area (Entzündungsherd und Entzündungshof) comes out very clearly. All simply hyperemic tissues become very pale, while the redness of the inflammatory focus undergoes almost no change. This demonstrates that blood-vessels within the focus lose the power to react to the effects

of suprarenal extract which otherwise affects normal vessels so readily. It is an instructive demonstration of, and a new proof for the theory that in inflamed tissues the blood-vessels lose many of their normal vital properties.

Now, in the course of these experiments we noticed in animals in which on one side the sympathetic nerve was cut and on the other side the ganglion was removed, that the inflammation on the sympathetic side was invariably greater than on the ganglion side. The cultures employed were the same for both sides, the quantities injected were as nearly equal as possible, and the places of injection were identical points in both ears. As in our previous experience the left ear has shown more pronounced vasomotor changes (after section of the sympathetic or removal of the ganglion) than the right we varied our experiments, operating in one animal the sympathetic nerve on the right and the ganglion on the left, and in another the sympathetic nerve on the left and the ganglion on the right side. Furthermore, in other experiments we have taken for each experiment two rabbits, operating the sympathetic nerve in one and the ganglion in the other animal; here the operation was performed in both rabbits on the same side — either the left or the right. In each animal the inoculations were then made either in both ears, having thus in each animal a comparison between the normal and the operated side; or the inoculations were confined to the operated ear. We can state now briefly that with one single exception the results were in all experiments the same, and that is that the inflammation on the side in which the sympathetic nerve was cut was regularly greater than that on the side where the ganglion was removed. The result was the same whether the inoculation was done soon after the operations or two or three weeks later. The experiments with turpentine have given the same results as those produced by the local infection. Furthermore, in the same animal after the first local lesion was healed or nearly healed, when inoculated again in another part of the ear or when a second lesion was produced by turpentine, the result was again the same, the sympathetic ear has shown the graver symptoms.

We have to say again that the differences were, of course, not so great as to be striking, especially when the inflammations in both ears were intense and covered a large area. By the use of our adrenalin method they could be made apparent even then, but the differences came out best when the lesion was small. The inflammatory process on the ganglion side was then sometimes very insignificant, while that on the sympathetic side was well pronounced, and led to the formation of a pustule.

When Claude Bernard¹ discovered the relation of the sympathetic nerve to the blood vessels of the ear, he set up at the same time the claim that after removal of the superior cervical ganglion the flushing of the ear is more vigorous and the temperature higher than after simple section of the sympathetic. This was soon contradicted by Schiff,² Becke van der Callenfels,³ and others, and the prevailing opinion of our time is not in agreement with that of Bernard. But assuming even that Bernard is right, and assuming further that the aggravating effect upon inflammation of the section of the sympathetic nerve or removal of the ganglion is due solely to the consecutive vasodilatation, to an increase in the blood supply, Bernard's opinion would be available as an explanation only, if the difference we have observed had been in the reverse direction, *i.e.*, if we would have found that after removal of the ganglion the inflammatory process is more flourishing; we could then have assumed that it is due to the greater vasodilatation, which occurs, according to Bernard, after this operation. But what we have found is just the reverse, namely, that after removal of the ganglion the inflammatory process is less vigorous than after section of the sympathetic nerve. If we then should hold that the relation of the nerves to inflammation is due exclusively to the vasomotor influences, we should have to assume, in order to make our observations intelligible, that while the sympathetic nerve is carrying vasoconstrictors which are normally in a tonic

¹ Claude Bernard. *Leçons sur la système nerveux*. Paris, 1858, ii, p. 492.

² Schiff. *Archiv für physiologische Heilkunde*, xiii, 1854, p. 523.

³ Callenfels. *Zeitschrift für rationelle Medizin*, 1855, p. 157.

state, the ganglion adds vasodilating fibers, the tonus of which becomes more apparent as soon as the constrictors are eliminated. The vasodilatation after section of the sympathetic would then be due to two factors: the elimination of the constrictor tonus and the activity of the vasodilating tonus emanating from the ganglion. When, on the other hand, instead of cutting the sympathetic nerve, the ganglion is removed, the vasodilatation coming from the ganglion becomes hereby eliminated and only the dilatation due to the elimination of the constricting tonus is left — hence the reduction in the inflammatory process after the removal of the ganglion, as compared with that occurring after the section of the sympathetic nerve. However, if this assumption were true, a lesser dilatation of the ear vessels ought to be seen after the removal of the ganglion than after simple section of the sympathetic nerve. As far as we know, this has not as yet been claimed by any one. It is true that in two or three experiments quoted by Becke van der Callenfels the flushing of the ear after removal of the ganglion appeared to be less than after section of the sympathetic. Callenfels quotes them, however, only to show the incorrectness of Bernard's claim of the greater effect of the removal of the ganglion, but does not draw the conclusion that the reverse is correct.

Moreover, in some of our experiments there was a distinctly greater vasodilatation on the side on which the ganglion was removed than on the sympathetic side. Nevertheless, the inflammation was more developed on the latter side. We have already quoted above our experience with the section of the third cervical nerve which favored the course of an inflammation very little, although the consecutive dilatation of the ear vessels exceeded that following section of the sympathetic nerve, or removal of the ganglion.

We are then compelled, it seems, to assume that the relations of the sympathetic nerve and the superior cervical ganglion to the course of inflammation, which we have observed in our experiments, are due to some other nervous functions of the sympathetic nerve and the ganglion than vasoconstriction and vasodilatation.

We offer the following provisional hypothesis: Many leading physiologists now hold the view that the metabolic processes of all tissues are under the control of antagonistic nerves: the anabolic nerves which have charge of the building up of the tissues, and catabolic nerves which control the breaking down of the tissues. Gaskel, for instance, considers the vagi as the anabolic and the accelerators as the catabolic nerves of the heart. The building up of tissue makes it, of course, more resistant and the breaking down makes it more susceptible to outside destructive influences. We offer, then, the suggestion that the sympathetic nerve carries the anabolic nerve fibers, and that from the superior cervical ganglion originate catabolic nerve fibers for the tissues of the ear. When the sympathetic nerve is cut, the favorable influence of the anabolic nerves is eliminated and the unfavorable effect of the catabolic nerves remains in activity; hence the greater susceptibility to inflammation. When the ganglion is removed, the detrimental activity of the catabolic nerves is abolished, and only the absence of the anabolic nerves remains as an aggravating factor; hence the lesser susceptibility after removal of the ganglion than after cutting of the sympathetic nerves.

The statements which we make in this paper appear to us to be of such importance that, though they are based on quite a large number of experiments, we feel that much more ought to be done before a final verdict can be given. We consider, therefore, our present report only as a preliminary communication.

However, some of the facts which we have stated in this paper have been sufficiently established in another line of investigation which we have carried out lately, and which we shall mention here in a few words. These facts are that nerve fibers can originate in the superior cervical ganglion and that they can have a function different and, in fact, the reverse from that which is exercised by the fibers of the sympathetic nerve. It has always been stated that the subcutaneous injection of suprarenal extract has no effect upon the blood pressure. We have found that a subcutaneous

injection of a medium dose of the extract causes in a normal rabbit a moderate but distinct dilatation of the blood-vessels of the ears. However, when the sympathetic nerve is cut on one side, the injection caused a constriction on this side every time, while the vessels of the other side became dilated. Furthermore, subcutaneous injection of the extract has no effect upon the pupil of a normal animal. Neither has instillation into the conjunctival sac any effect. Nor has either of these methods of administration any effect upon the pupil when the sympathetic nerve is cut; but when the entire ganglion is removed, twenty-four hours after the operation a subcutaneous injection or an instillation will cause in a few minutes an ad maximum dilatation of the pupil. That means that the nerve fibers originating from the ganglion inhibit the dilatation of the pupil. Now we know that the nerve fibers within the sympathetic nerve favor the dilatation of the pupil. The nerve fibers of the ganglion and the sympathetic nerve possess, then, antagonistic activities.

We have to add that the injection of adrenalin fails to affect the pupil if only a part of the ganglion has been removed, even if this part be more than half. This is a point which has to be kept in mind in cases of failure in the adrenalin experiments as well as in the experiments on inflammation.

THE PATHOLOGICAL CHANGES IN THE NERVOUS SYSTEM IN A
CASE OF LEAD POISONING.*

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Although paralysis occurring in lead poisoning has been recognized many years, the pathology of lead palsy and lead encephalopathy has not been fully determined. A case with very positive symptoms has occurred in the service of Dr. F. P. Henry, at the Philadelphia Hospital, and the material obtained from this case at necropsy very kindly has been given to me for study by Dr. Henry. The clinical notes are his.

P.S., a man, aged forty-eight years, was admitted to the Philadelphia Hospital, Jan. 16, 1902, complaining of abdominal pain and weakness in the extremities. He had been working in lead about twenty years, and had had attacks of lead colic.

During the four weeks previous to admission he had had pain in the abdomen and back, which had become gradually worse, and weakness had been increasing during this time. On admission he was poorly nourished. The irides reacted to light and in accommodation. The conjunctiva was inflamed, and there was a muco-purulent discharge from the eyelids; the tongue was protruded in the median line. A characteristic blue line was found on the gums. The pulse was regular and of fair volume. The arteries were moderately sclerotic. The abdomen was scaphoid. The cardiac impulse was felt in the fifth interspace. The dulness was normal in extent. The valvular sounds were well defined, and the second aortic was accentuated. The lungs appeared to be normal. The liver was diminished in size and the spleen was not palpable. Slight wrist-drop was present. The patient could supinate and pronate the hand to a limited extent; the grip was feeble and he could not lift the arms. The reflexes were absent. The lower limbs were completely paralyzed. The plantar reflexes alone were retained. The Babinski reflex was not obtained. Sensation generally was preserved.

* Read at the meeting of the American Association of Pathologists and Bacteriologists, May, 1903. Received for publication, June 1, 1903.

On January 19 the paralysis in the upper limbs was more complete than on admission.

On January 21 a note was made that the man was delirious at times, especially at night, and on January 30 the delirium was still very great.

The urine contained much albumin and casts.

On February 5 it was noted that he had incontinence of urine. He was still delirious at night and occasionally during the day. The paralysis had largely disappeared in the lower limbs, but marked wrist-drop was present.

The blood count during the height of the disease was as follows: white cells, twenty-one thousand, red cells two million five hundred thousand, hemoglobin forty per cent. Red cells in staining gave the degenerative granular change.

The report of Dr. C. A. Oliver regarding the ocular condition was as follows: "Feb. 4, 1902, Marked catarrh from the left conjunctiva. The pupils are 3 mm. in diameter. The irides are very sluggish, especially the right. There is rather high grade retinitis without any hemorrhages, more pronounced in the right eye."

On March 15 the paralysis in the upper limbs was unaltered. The lower limbs could be moved at will. Diarrhea existed.

The patient died March 19, 1902.

At the necropsy fibrosis of liver and spleen was found. The kidneys were small and there was considerable interstitial change. The myocardium appeared degenerated, but the cardiac valves were not markedly altered.

Right Paracentral Lobule: The blood vessels in the pia and in the substance of the brain are much congested. Within the cortex there seems to be an increase of neuroglia cells. Small accumulations of round nuclei are found about some of the blood vessels of the cortex, and amyloid bodies are found within the cortex just below the pia. The pia in some places is intimately adherent to the cortex. Recent hemorrhages are found within the pia and between the pia and the cortex. Considerable round-cell infiltration is found within the pia.

Sections from the left paracentral lobule resemble closely those from the right paracentral lobule. The cells of Betz, even by the thionin stain, are not intensely altered, although in some there is displacement of the nucleus and partial disintegration of the chromophilic elements, but, as compared with the

cell-bodies of the anterior horns of the spinal cord, they are remarkably well preserved. Most striking is the proliferation of the endothelial cells at places on the outer surface of the pia, with the formation of masses or long rows of cells upon the surface of the pia. This proliferation of endothelial cells is most pronounced in the motor region of the cortex, but is distinct also in sections from the right frontal lobe, right parietal and right occipital lobes.

Sections from the right optic thalamus do not present any proliferation of the ependymal cells.

Sections from one of the cerebellar lobes do not show distinct pathological changes.

Marchi sections from the cervical and lumbar regions of the spinal cord present no degeneration of the white matter, but accumulations of black dots are found along the intramedullary portion of the anterior roots. These accumulations are not very pronounced, but they are possibly indicative of degeneration of the medullary sheaths. The anterior and the posterior roots of the lower cervical region, even when cut separately from the spinal cord and stained, do not appear to be degenerated. The nerve cell-bodies of the anterior horns in the cervical and lumbar regions are intensely degenerated; the alteration consisting of displacement of the nucleus, chromatolysis, vacuolation of some of the cell-bodies, and pigmentation. The alteration is fully as intense in the lumbar region as in the cervical, and all the groups of cell-bodies are affected. The posterior roots of the lumbar region show little alteration.

A portion of one of the peripheral nerves (median) stained by the Marchi method presents intense degeneration. Stained by the Weigert hematoxylin stain, the same nerve shows considerable degeneration. Pieces of the median and sciatic nerves alone were saved for examination.

The sciatic, by the Weigert hematoxylin stain, does not present much alteration.

Sections from a muscle on the anterior surface of the forearm, and from the pronator radii teres, show a considerable increase in the number of nuclei between the muscle fibers,

giving the appearance of interstitial myositis. The muscle fibers are not much atrophied, and black dots within the muscle fibers, showing fatty degeneration, are not found by the Marchi method. Nerve bundles within the muscles are much degenerated. Here and there an unusually large muscle fiber may be found.

Sections from one of the lumbar or sacral spinal ganglia show proliferation of the endothelial cells of the capsules, without much interstitial round-cell infiltration. The condition resembles closely that seen in rabies.

The views entertained regarding the pathological changes in lead palsy are clearly presented by Remak and Flatau in their monograph on neuritis. According to them, only a few investigators (Hitzig, Harnack, Friedländer) believe that the muscles are primarily diseased, and most investigators hold that the lesions are primarily in the peripheral motor nerve fibers, but it is uncertain whether the lesions are primarily in the peripheral nerve fibers, or in the cell-bodies of these nerve fibers. The supporters of the peripheral theory (Westphal, Charcot, v. Leyden, Schultze, Eisenlohr, and others) advance as an argument the occurrence of degenerative changes in the peripheral nerves, while the anterior spinal roots and the spinal cord remain intact. Erb and Remak have suggested that lead may cause a functional disturbance of the nerve cell-bodies, not detectable by the microscope, and that this in turn may cause the degenerative changes in the peripheral nerves. Pathological changes, however, have been detected in the spinal cord and anterior roots, but not in the majority of cases.

The degenerative changes are said to be usually most pronounced in the posterior interosseous nerve, though not confined to this nerve, and they diminish in intensity toward the proximal end of the nerves.

The only investigators who have found changes in the spinal cord of man in lead paralysis, mentioned by Remak and Flatau, are Vulpian, Oppenheim, v. Monakow, Oeller, Zunker, and Goldflam. Such changes, however, in experimentation by

Stieglitz, by Schaffer, and by Nissl are referred to by them. In the thesis of Mme. Dejerine-Klumpke on polyneuritis and lead palsy in particular, published in 1889, changes in the spinal cord occurring in lead palsy are said to have been found only in five cases (Vulpian, v. Monakow, Zunker, Oeller, Oppenheim), so that the only additional name mentioned by Remak and Flatau is Goldflam. Mme. Dejerine-Klumpke remarked that the existence of lesions in the muscles and peripheral motor nerves in lead palsy was well known and beyond all dispute, while it was very different as regards spinal lesions. In Vulpian's case, some of the nerve cells of the spinal cord had a colloid or vitreous appearance; in von Monakow's case, in the spinal cord were foci of sclerosis, alteration of the walls of the vessels, small hemorrhages and accumulations of lymphatic cells in the pericellular spaces, and the lesions as well as the symptoms resembled those of parietic dementia; in Zunker's case, the number of nerve cells was diminished; in Oeller's case, foci of hemorrhage and softening were found, and the nerve cells were small and stained badly; and in Oppenheim's case, many of the nerve cells had disappeared. This last case, according to Mme. Dejerine-Klumpke, is the only one of the five in which the lesions of the cells of the anterior horns were very positive. Vitreous change or diminution in the number of nerve cells is of doubtful value, and capillary hemorrhages are not uncommon in cases of interstitial nephritis, such as existed in Oeller's case.

The case that Goldflam reports was extraordinary on account of the pathological changes in the nerve cells of the anterior horns, and on account of degeneration of nerve fibers of both gray and white matter and of anterior spinal roots. Many of the intraspinal vessels had thickened walls. The alteration was more intense in the cervical than in the lumbar region, and yet the paralysis in the upper and lower limbs was equal. Although these changes in the spinal cord were so pronounced, Goldflam favors the peripheral theory.

Carlo Ceni found the nerve cells of the anterior horns of the spinal cord very much degenerated, the pigment was

much increased in amount, nuclei of nerve cells were displaced or absent, and the cellular processes were imperfect.

Laslett and Warrington have observed pathological alteration of the nerve cells of the anterior horns in the cervical region, and atrophy of nerve fibers in the anterior roots of the same region in a case of lead palsy in man.

F. Quensel has found much alteration of the nerve cells in the anterior horns of the lumbar and cervical regions of the spinal cord in a case of lead poisoning in man.

In a case of lead palsy observed by Philippe and Gothard the nerve cells of the anterior horns were less numerous than normal and some were atrophied, the vessels were sclerotic and the neuroglia slightly proliferated. The anterior roots were degenerated. Peripheral nerves examined were degenerated secondarily to the lesions of the cells in the anterior horns. Muscles were atrophied. The authors believe this case offers support for the central theory.

I know of no other cases of lead palsy in man with degenerative changes in the nerve cells of the spinal cord. To the cases already mentioned may be added the one reported by me in this paper.

In a case of lead palsy with unconsciousness, examined by Carlo Ceni, the brain was edematous, as it was especially in a case reported by Chvostek.

The edema of the brain described by Ceni, Chvostek, and others, in lead palsy, possibly may explain the rapid development of cerebral symptoms, even focal in character, as in the case of hemiplegia developing in acute lead poisoning reported by J. M. DaCosta. DaCosta refers to the examinations of Maier, which show that in the brain lead has a special affinity for the cortex. Heubel has found by experimentation that next to the liver and spleen the relatively greatest amount of lead is found in the brain and spinal cord. (Cited by Quensel.)

I have asked Dr. D. L. Edsall to make an examination of the brain for lead in my case, and the following is his report:

"A portion of the cortex about an inch square was first taken, and was completely oxidized by Neumann's method (heating in a mixture of

sulphuric acid and nitric acid). The fluid was then partly neutralized with ammonia and filtered. To the filtrate ammonia was added until the reaction was only slightly acid. The residue on the filter was boiled with concentrated hydrochloric acid, and this was filtered while hot. No precipitate occurred on cooling. To this ammonia was added until the reaction was only slightly acid. H_2S was then run through both the fluids for a long time. Not the slightest precipitate was produced.

"I then carried out the same procedure with about one-third of the cerebral hemisphere that was furnished me. The result in this instance, also, was entirely negative. A large portion (about one-third) of the hemisphere was then partly oxidized with nitric acid and subsequently incinerated. The ash was boiled with concentrated hydrochloric acid, and repeatedly extracted in this way. The extract was tested for lead, with entirely negative results. As far, then, as I was able to determine, there was no lead present in the cerebral hemisphere furnished me."

Dr. Edsall's failure to obtain lead from the brain does not throw any doubt upon the diagnosis of the case, as in a number of cases of lead poisoning, as shown by Quensel, lead could not be obtained from the brain, and it has been supposed that in these cases the lead affected the brain indirectly by some alteration of the blood or some form of autointoxication.

The reports of microscopical investigations of the brain in man in lead palsy, even with encephalopathy, are not numerous.

The alteration of the nerve cells of the cerebral cortex in Ceni's case was most intense in the frontal lobe.

In a case of lead poisoning in man, F. Quensel has found very pronounced changes in the brain. The pia arachnoid was thickened, its vessels were very numerous and their walls were thickened, and recent small hemorrhages were found between the pia and cortex. In the cortex the nuclei in the walls of the vessels were poliferated, and the vessels were surrounded by accumulations of gliar cells. Deiters' cells were numerous. The nerve cells were much altered in the cerebral cortex. Quensel gives numerous references to the literature on lead palsy, and refers to cases of lead encephalopathy with necropsy. Most of these are of questionable value, and many of the examinations were only

macroscopic, and the lesions were such as edema, or anemia. The cases of lead poisoning with symptoms of parietic dementia cannot be studied to determine the lesions of lead, because similiar lesions are found in parietic dementia without lead, and the changes caused by the lead alone are uncertain. Quensel refers, however, to two cases of lead poisoning with cerebral changes, although encephalopathy did not exist in either one, in which microscopical examination was made. One was reported by Kussmaul and Maier, the other by Ceni, already referred to. The former was reported in 1872, and according to Quensel there was increase of connective tissue about the vessels, and the smaller vessels were not as large as normal. In Ceni's case the brain was atrophied, edematous, and anemic, the processes of the nerve cells appeared varicose by the Golgi stain, and the nerve cells showed fatty degeneration by the Marchi method. The walls of the vessels were not thickened, and there was not much alteration of the vessels. Quensel's case seems to be the only thoroughly satisfactory one of lead encephalopathy with microscopical examination.

The case that I report, therefore, is a contribution to this subject, little studied from a pathological aspect.

I make mention especially of the proliferation of the endothelial cells upon the surface of the cerebral pia. I have not found mention of such an occurrence in man or in the lower animals.

Stieglitz found cerebral changes in some of his animals poisoned by lead, and there were numerous smaller or larger hemorrhages about the vessels and cellular infiltration about some of the vessels.

In the brains of dogs poisoned by the acetate of lead, McCarthy has found changes more marked in the cortex around the gyrus cruciatus which corresponds to the motor area in man. The nerve cells were degenerated, the capillaries of the cortex were increased in number, and their walls were thickened and were surrounded by accumulations of cells. Small hemorrhages were found in the cortex. McCarthy's examination was confined to the brain,

Stieglitz in his experimental poisoning of animals with lead frequently found the posterior roots more or less degenerated. He remarks that even in man sensory fibers of the nerves must be affected, or else it would be difficult to explain the arthralgia which is so frequent that Tanquerel found it in 755 cases out of 2,151 (35 per cent). The degeneration in the posterior roots he found confined almost to the medullary sheaths. He remarks also that the posterior roots in man have seldom been examined in cases of lead palsy, but that Schultze found in one case a small atrophic focus in a posterior cervical root.

I have found the posterior roots of the cervical region normal, as well as those of the lumbar and sacral regions, except that the latter contained a few swollen axis cylinders.

Stieglitz found in the spinal ganglia proliferation of the connective tissue and shrinkage and vacuolation of the nerve cells from lead poisoning.

In one of his animals made to inhale lead during a period of six days, and in another during a period of eight days, the posterior and anterior spinal roots contained degenerated medullary sheaths. Numerous hemorrhages were found in the spinal cord, especially in the anterior horns and central gray matter, and the nerve cells of the spinal cord were vacuolated. These findings are especially interesting because of the rapidity of the poisoning. He refers to three cases in man reported by Tanquerel des Planches, in which palsy developed eight days after the first exposure to lead. In that reported by Da Costa, the development of the palsy occurred very soon after the exposure to lead.

Philippe and Eide have found very distinct changes in the nerve cells of the spinal ganglia in one instance of lead poisoning. The nerve cells were small and shrunken and much pigmented. They do not speak of round-cell infiltration in the ganglia in this case.

In the case that I report in this paper, changes were found in the spinal ganglia very much like those of rabies. There was distinct proliferation of the endothelial cells lining the capsules of the nerve cells, and this was evident where

there was little round-cell infiltration. In a paper read before the Pathological Society of Philadelphia, Dec. 27, 1900, I reported the finding in two cases of lesions resembling those of rabies. Neither case was one of rabies. I had no desire to diminish by this report the importance of microscopic examination in suspected cases of rabies, but I merely wished to show that one must be cautious in making a diagnosis of rabies from the lesions alone. In my case of lead poisoning, the proliferation of the endothelial cells lining the capsules in the spinal ganglia is like the proliferation of the endothelial cells on the surface of the cerebral pia, and may show that lead has an affinity for endothelial cells. I do not know whether endothelial cells elsewhere in the body were proliferated.

Changes in the muscles have been observed in lead palsy. In Ceni's case, muscle fibers were degenerated and the nuclei in the interstitial tissue were increased in number. Madame Dejerine-Klumpke also has described such alteration of the muscles, and it is present in this case I report, in which there is an increase of the interstitial cells.

I conclude, therefore, from my study of this case and of the literature that lead affects the brain and its pia, the nerve cells of the anterior horns of the spinal cord, the ganglia on the posterior roots, the peripheral nerve fibers, and the muscles. It seems to be impossible to determine whether its effects are first manifested in alteration of the peripheral motor fibers or of the motor cell-bodies of the spinal cord, but inasmuch as both peripheral motor fibers and motor nerve cell-bodies are sooner or later affected, this question is not a very important one. The significance of the proliferation of the endothelial cells of the capsules in the spinal ganglia and on the cerebral pia is difficult to determine.

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PLATE I.

- Fig. 1. Proliferation of endothelial cells upon the surface of the cerebral pia.
- Fig. 2. Degeneration of nerve cells of the anterior horns of the spinal cord.
- Fig. 3. Degeneration of the median nerve, one per cent osmic acid.
- Fig. 4. Cellular infiltration between muscle fibers.

ON AN ABNORMAL THORACIC DUCT.¹

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Interest in the anatomy of the thoracic duct is perhaps limited, and even with the importance which surgery sometimes lends, the duct itself rarely calls for much attention. Yet, from time to time, marked variations or rare anomalies are reported, which may serve as interesting anatomical or valuable surgical facts.

The workers of the eighteenth and nineteenth centuries have done much which cannot be displaced nowadays in patient detail and accurate description on this subject. Such results as those of Cruikshank, Mascagni, Breschet, and Sappey have perhaps added most to this knowledge, with the earlier pioneer work of Pecquet.

In the course of an investigation to determine the frequency of variation, as well as several points of surgical anatomy, of the thoracic duct, one or two cases of irregularity have been found. Two of these seemed of enough value to report here. Both occurred in male subjects, were found in the dissecting room, and the ducts were injected from the receptaculum with hot wax.

Of the normal anatomy this only need be said: The thoracic duct begins as an enlargement at the upper lumbar vertebræ, anterior to the spine, and between the aorta and azygos major vein. Passing upwards, it extends as a single vessel, to end by opening in the angle formed by the left subclavian and internal jugular veins. The duct rises, however, in the neck, usually not higher than the upper level of the seventh cervical vertebra.

Of the two instances of irregularity of the thoracic duct referred to, the first is the occurrence of a division of the duct, beginning at the level of the third thoracic vertebra,

¹ Received for publication June 4, 1903.

these two branches not uniting again before emptying into the veins in the neck. Both branches were of equal size, and were separated from each other by connective tissue and fat. The normal anatomical and surgical relations existed for the higher of these two branches, even in the cervical region; and the lower branch passed up and out in close relation with the subclavian artery. Such division as this is probably not rare, and is reported to call attention again to the actual condition rather than on account of any curiosity.

The second case is the occurrence of both right and left duct. No other anatomical abnormalities were noted; and the large blood vessels arising from the aorta were normal in arrangement. Beginning at the receptaculum on the first and second lumbar vertebræ, the duct passed up as a single vessel to the level of the seventh thoracic vertebra, where it broke up into a plexus of small branches. So far the duct had normal relations, lying between the aorta and azygos major vein, anterior to the intercostal vessels. From the level of the sixth to eighth thoracic vertebræ, two branches passed up and to the left, behind the esophagus, left bronchus, and arch of the aorta to the level of the second thoracic vertebra. The two branches united then for the distance of an inch, only to divide again. One of these passed into the neck to the level of the fifth cervical vertebra, where it turned outward and downwards, to break up into three or four smaller branches; and so opened as a delta, into the junction of the great veins on the left.

At the level of the sixth to eighth thoracic vertebræ where, as already mentioned, branches passed up and to the left, other branches passed up, but to the right, forming a considerable anastomosis about the right bronchus; these branches, six in number, passed along the right of the trachea to the level of the seventh cervical vertebra, where they united to form one good-sized duct. As the left thoracic duct rose in the neck abnormally high, so on the right the duct rose to the level of the "carotid tubercle" of the sixth cervical vertebra, where it turned outwards and downwards

to empty by two branches of unequal size into the confluence of the internal jugular and subclavian veins on the right.

In this case these points of interest are to be noted: first, at the level of the sixth to eighth vertebræ the formation of a plexus of branches from the single main duct; second, the presence of both left and right thoracic ducts; third, the unusual height to which both rise in the neck, — that on the left passing to the level of the middle of the thyroid cartilage or the body of the fifth cervical vertebra; and fourth, the absence of abnormality in the great arterial trunks or arch of the aorta.

Such work as has been done on the thoracic duct in lower vertebrates has shown that not infrequently it may be double, or otherwise irregular. In the horse, in some fishes, in the pig, in man, the duct has been found double, one branch passing to the right, the other to the left side, to empty into the veins. And it is reasonable, therefore, to look to comparative anatomy on the one hand, and to embryology on the other, for an explanation of the abnormal conditions in the human being. Until recently, however, little work on this point has been done, but Ranvier, in France, and Sabin, in America, have done something to clear up the development of the thoracic duct. From their researches it is known that in the pig the lymphatic system develops from the cardinal veins in a bilaterally symmetrical way. At first four lymph-hearts, two anterior, two posterior, appear; and from these later develop the right and left thoracic ducts, as well as a right and a left receptaculum chyli. The left thoracic duct, in relation with the development and persistence of the left aortic arch, forces itself caudad in a more vigorous fashion than the right, so that the latter passes only to the right lung.

According to these researches, then, a main lymph channel develops in connection with, or corresponding to, each primitive aorta. The bilaterally symmetrical cardinal veins are also in important relation with the beginnings of these main lymph channels. It would, therefore, be natural for

abnormalities in the primitive aortic arches, either with transposition of the viscera or with an abnormal origin of the large arterial trunks, as the subclavian, to cause irregularities in the thoracic duct. This seems to be usually the case, as shown by the frequent occurrence of a right thoracic duct with an abnormal origin of the right subclavian artery. In regard to the second case reported here, the right and left primitive lymph channels have, without doubt, persisted and united to form in the inferior portion a single trunk.

NOTE. — My thanks are due Professor Dwight, of the Harvard Medical School, for use of anatomical material.

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CONTENTS.

	PAGE
A STUDY OF THE AGGLUTINATING, HEMOLYTIC, AND ENDO- LYOLYTIC ACTION OF THE BLOOD SERUM IN VARIOLA.	
<i>W. T. Howard, Jr.</i>	157
STUDIES ON THE ETIOLOGY AND PATHOLOGY OF VARIOLA.	
<i>R. G. Perkins and G. O. Pay</i>	163
STREPTOCOCCUS PYOGENES IN VARIOLA.	
<i>R. G. Perkins and G. O. Pay</i>	180
BACTERICIDAL ACTION OF THE BLOOD SERUM IN VARIOLA AND IN VARIOLOID.	
<i>R. G. Perkins and G. O. Pay</i>	196
ON THE CHEMISTRY OF THE CHROMATIN SUBSTANCE OF THE NERVE CELL.	
<i>P. A. Levene</i>	204
ON THE AUTOLYSIS OF BRAIN TISSUE.	
<i>P. A. Levene and L. B. Stookey</i>	212
ON THE DIGESTION AND SELF DIGESTION OF TISSUES AND TISSUE EXTRACTS.	
<i>P. A. Levene and L. B. Stookey</i>	217
THE AGGLUTINATION OF THE PNEUMOCOCCUS WITH CERTAIN NORMAL AND IMMUNE SERA.	
<i>Augustus Wadsworth</i>	228
A PATHOLOGY FOR FORAGE POISONING, OR THE SO-CALLED EPI- ZOÖTIC CEREBRO-SPINAL MENINGITIS OF HORSES. (With one plate.)	
<i>D. J. McCarthy and Mazyck P. Ravenel</i>	243
CAT'S BLOOD — DIFFERENTIAL COUNTS OF THE LEUCOCYTES. (With one plate.)	
<i>F. C. Busch and Charles Van Bergen</i>	250
THE REACTIONS OF THE BLOOD IN EXPERIMENTAL DIABETES MELLITUS.	
<i>J. E. Sweet</i>	255
THE PATHOLOGY OF CHRONIC FLUORINE POISONING.	
<i>Fritz Schwyzer</i>	301

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No. 2.

A STUDY OF THE AGGLUTINATING, HEMOLYTIC, AND ENDO- THELIOLYTIC ACTION OF THE BLOOD SERUM IN VARIOLA.*

WILLIAM TRAVIS HOWARD, JR., M.D.

(Professor of Pathology, Western Reserve University.)

(From the Small-pox Reserve Laboratory of the City of Cleveland and the Pathological
Laboratory of Western Reserve University, Cleveland.)

The object of this research was to determine the action of the blood serum in variola upon: (I.) normal human erythrocytes and leucocytes from (*a*) vaccinated, and (*b*) unvaccinated individuals; (II.) erythrocytes of individuals with variola; (III.) leucocytes of pus in a non-variolaous individual; (IV. and V.) erythrocytes of the rabbit and ox; (VI.) vascular and serous membrane endothelium of man and the rabbit.

Methods and technic. — All the experiments were made under the same conditions and with the same technic. My thanks are due to Dr. R. G. Perkins and Mr. G. O. Pay for obtaining the blood serum. Blood was obtained in some cases from the arm during life, but in most it was aspirated a short time after death from a jugular vein or the heart, placed on ice in sterile vessels until the separation of the serum. The latter was kept on ice in sterile sealed vessels.

As will be pointed out in another paper by Dr. Perkins and Mr. Pay, the blood in these cases usually contained streptococci in larger or smaller numbers. The sera used in my experiments were always free from organisms and remained

* Read at the meeting of the American Association of Pathologists and Bacteriologists, Washington, D.C., May 13, 1903. Received for publication July 10, 1903.

clear and active as long as three months after removal from the body. There was no evidence of multiplication of streptococci in the sera. Owing to the small amount of serum available in some cases, small and uniform quantities were used in all the experiments. A mixture of 0.4 cc., of 0.85 or 0.9 per cent, 0.1 cc. of serum, and 0.01 cc. of washed or unwashed cells were used.

The test-tubes and pipettes were sterile. The results were noted immediately and after fifteen minutes at room temperature, after two hours at body temperature, and after twelve and twenty-four hours in the ice-chest.

A synopsis of the cases from which the sera were obtained is given below:

Case I. Male, white, age 2 years; death from variola hemorrhagica pustulosa on the sixth day of eruption. General infection with streptococcus.

Case II. Male, white, age 22 years; dead with variola confluens on the tenth day. General streptococcus infection.

Case III. Male, age 30 years; dead of variola confluens on the seventh day. General streptococcus infection.

Case IV. Male, white, 40 years; recovered from variola confluens of a mild type. Blood aspirated from arm on the twenty-first day. Cultures made from blood during illness showed no streptococci.

Case V. Female, age 3 years; dead of variola purpura on the first day of eruption. No streptococci obtained.

Case VI. Female, age 19 years; dead of variola confluens on sixth day of illness. Blood obtained at autopsy immediately after death contained streptococci and staphylococcus aureus.

Case VII. Female, age 20 years; dead of streptococcus infection after variola confluens.

Case VIII. Male, age 39 years; dead of variola hemorrhagica pustulosa on the fifth day of the disease. Streptococci were present in the blood and organs.

The fact that human blood serum in a number of diseases contains both isoagglutinins and isohemolysins has been well established by a number of observers during the last three years. Shattock,¹ in 1900, observed marked isohemagglutination of the blood serum in pneumonia, erysipelas, and acute articular rheumatism; while Landsteiner,² in 1900, found that

the serum of all of twenty-two healthy adults had an isoagglutinating action for red blood cells. Eisenberg,³ in 1901, found that of ten healthy adults the serum of only one contained isoagglutinin. He examined the blood of one hundred and forty persons with various diseases (including typhoid fever, scarlatina, syphilis tuberculosis, pneumonia, diphtheria, and nephritis) and found that seventy-five contained isoagglutinin. According to the observations of De-castello and Sturli,⁴ the blood of most persons, both healthy and ill, over six months of age contains isoagglutinin. Indeed, of the one hundred and fifty-one cases studied by them, isoagglutinin was absent in only one of thirty-four healthy individuals, and in three of one hundred and thirty-four sick. Herter,⁵ in 1902, observed that the blood of nearly one-third of the patients of a certain hospital contained isoagglutinin. The occurrence of isohemolysins in the blood in various conditions is too well known to call for reference here.

I. (a.) Washed erythrocytes of two healthy vaccinated men + variola serum.

TABLE I.

SERUM.	AGGLUTINATION.		HEMOLYSIS.
	Erythrocytes.	Leucocytes.	
Case I.	+ rapid, marked.	+	o
" II.	+ " "	+	o
" III.	+ " "	+	o
" IV.	+ " "	+	o
" V.	+ not rapid, marked in two hours.	+	o
" VI.	+ as above.	+	o
" VII.	+ moderate in 15 minutes, marked in two hours.	+	o
" VIII.	+ rapid, marked.	+	None after two hours in the in- cubator, but marked after 46 hours on ice; the other tubes under same conditions showed no lysis of the cells.

The agglutination was staphylic and often with poikilocytosis. Filtration through a porcelain filter did not affect the activity of the serum (Case V.). Sera I., II., and III., heated to 56° and 59° C. for one hour, were as active as before heating. Sera III. and VII., both heated and unheated, agglutinated unwashed as readily as washed corpuscles. Agglutination was followed more or less rapidly by sedimentation of the corpuscles.

(b.) Erythrocytes and leucocytes (washed and unwashed) of unvaccinated children + heated and unheated variola serum.

TABLE II. — WASHED ERYTHROCYTES AND LEUCOCYTES + UNHEATED SERUM.

SERUM.	AGGLUTINATION.	
	Erythrocytes.	Leucocytes.
Case III.	+ rapid, marked	?
“ VII.....	+ “ “	?
“ VIII.	+ “ moderate	?

Crenation and poikilocytosis occurred with Sera III. and VII.; agglutination was staphylic in all three. There was no hemolysis. Heating to 59° C. caused no loss of agglutinin. These sera also agglutinated, without hemolysis, the unwashed erythrocytes of unvaccinated children.

II. Erythrocytes and leucocytes of an individual with variola + variola serum. Sera III. and VII., unheated, caused rapid agglutination of both washed and unwashed erythrocytes. The agglutination was staphylic without poikilocytosis. Serum III. also caused hemolysis of washed erythrocytes. Agglutination without hemolysis was obtained upon washed and unwashed erythrocytes of this case with Sera III. and VII., heated at 58° C. for an hour.

III. The effect of variola serum upon the washed leucocytes of a streptococcus abscess of a non-variolaous vaccinated man.

Nearly all the cells were polymorphonuclear leucocytes. Three sera (numbers III., V., and VI.), both heated to 59° C. and unheated, caused neither agglutination nor leucolysis. This experiment was suggested by the statement of Councilman (since confirmed by Perkins and Pay in these laboratories) that a marked leucolysis occurs in the blood vessels in variola. I was unable to demonstrate this outside the body in either the test-tube or the hanging drop.

IV. The effects of variola serum upon washed and unwashed rabbit's erythrocytes. Sera III., VII., and VIII. caused rapid and complete agglutination (staphylic), without hemolysis, of both washed and unwashed erythrocytes. Heating to 59° C. caused no loss of agglutinin.

V. Effects of heated and unheated variola serum upon washed and unwashed erythrocytes of the ox. Sera III. and VII. caused neither agglutination nor hemolysis.

VI. Effects of variola serum upon endothelium.

By this experiment it was hoped to find the explanation of the hemorrhage and evident vascular lesions (swelling and disintegration of the vascular endothelium) in variola. Washed and unwashed vascular and serous membrane endothelium of the rabbit, and washed and unwashed vascular endothelium of man suspended in salt solution + the serum of Case V. (variola purpura) were examined in the test-tube and in the hanging drop under the microscope. Endotheliolysis was not observed.

CONCLUSIONS. — I. The blood serum of variola obtained from both fatal and non-fatal cases, early and late in the disease, with and without streptococcus mixed infection, causes agglutination of washed and unwashed erythrocytes of vaccinated, unvaccinated, and variolous human beings and of the rabbit.

II. This agglutinating property is not affected by heating to 59° C. for one hour.

III. Variola serum has little, if any, hemolytic action upon the erythrocytes of normal human beings. The washed erythrocytes of a variolous patient were dissolved by one of two variola sera tested.

IV. As variola serum is apparently inactive upon the washed and unwashed endothelial cells of the rabbit and man, it is probable that the lysogenic effect exerted on the vascular endothelium in this disease is a local one.

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STUDIES ON THE ETIOLOGY AND PATHOLOGY OF VARIOLA.¹

ROGER G. PERKINS, M.D., AND GEORGE O. PAY.

(From the Small-pox Research Laboratory of the Health Department of Cleveland, and the Pathological Laboratory of Western Reserve University, Dr. W. T. Howard, Jr., Director.)

In the course of the severe epidemic of small-pox which broke out in Cleveland in 1902, opportunity was given us to study the disease under very favorable conditions. The laboratory was made possible through the efforts of Dr. W. T. Howard, Jr., and it is our pleasant duty to here express our gratitude for his energy in procuring the necessary funds and permissions, and for his advice and assistance throughout the work. The laboratory was established in the Detention Hospital, and was kept in commission from the end of October, 1902, until the first of April, 1903, at which time it was closed for lack of cases.

Inasmuch as it was clearly not possible for two men to take up so large a problem from all points of view and to do justice to any of them, we had to be guided in our selection by the opportunities at hand.

As far as we could see at that time, the pathological and histological end had been very thoroughly worked up, and it was furthermore obvious that the material obtained in the course of our residence could be used at any time after, if properly fixed and preserved.

For the last twenty-five years the so-called *vaccine bodies* have been seen and noted, first in vaccine and later in variola, both in the affected tissues and in the fresh vaccine. The discussion has been active, Guarnieri, Pfeiffer, and their school considering them as undoubted animal parasites, while others attribute their origin to other sources. Ferroni and Massari considered them as derived from the nuclei of epithelial cells, Huckel as of cytoplasmic origin, and of late

¹ Received for publication July 29, 1903.

NOTE.—The work embodied in this paper and the two that follow was made possible by a special Research fund of \$4,000 granted by the City of Cleveland for the study of the Etiology and Pathology of Variola.

Ewing¹ has suggested broken-down erythrocytes as the origin of at least a part of the forms seen in vaccine. Still more recently Borrel² has described cell inclusions in sheep-pox, molluscum contagiosum, cattle plague, and epithelioma of mice, which he considers to be very similar to those seen in variola and in vaccinia, and which he attributes, not to stages in the development of parasites, but rather to the invasion and breaking down of leucocytes. In view of this diversity of opinion, we decided to postpone examination and experimentation dealing with stained and hardened material, and to confine our efforts to the fresh material, which could be used only during the persistence of the epidemic.

Review of the literature on the subject seemed to us to leave little possibility that the disease was due to any of the known bacteria, or to other bacteria in any way similar. The absence of such organisms in the lesions, as noted by others and confirmed by us in another paper,³ together with the lack of confirmation of the claims of those who found bacteria in variola and claimed them to be the cause, seemed sufficient to exclude these micro-organisms. As to the possibility that the organism might belong to the group of so-called sub-microscopic bacteria, the experiments of Park,⁴ showing that filtered vaccine is inactive, indicate that the infectious agent is too large to pass through the ordinary Chamberland and Berkefeld filters. In another series of experiments Wherry⁵ found that a small, active bacillus which he isolated from a case of pneumonia in a guinea-pig was able to pass readily through a Berkefeld 5, though not through a Berkefeld 8. He also found that the colon bacillus was able to grow through the Berkefeld 8, if allowed to stand at incubator temperature for twenty-four hours. Thus it is probable that the vaccine and variola organisms are, at the very least, within the limits of vision. If, then, these diseases are caused by an organism belonging among the bacteria, it must be one

¹ Proc. N.Y. Path. Soc., ii, No. 4.

² Annales de l'Inst. Pasteur, Tome xvii, p. 81.

³ Streptococcus pyogenes in variola. This Journal, this number, p. 180.

⁴ Proc. N.Y. Path. Soc., ii, 71.

⁵ This Journal, viii, 322.

which is in many essentials entirely different from any which we have as yet discovered. The special relations of *Streptococcus pyogenes* to variola have been discussed by us in a separate paper.¹

Given this improbability of bacterial origin, and the comparative assurance that the organism must be large enough to see, the best place to look for it seemed to be the exudate, especially in the earlier stages, and, perhaps, also the circulating blood.

The literature on the contents of the vesicles and pustules is quite large, and embodies a variety of opinions. Guarnieri confined his attentions to vaccine, and there described the well-known *Cytoryctes vaccinæ*. Pfeiffer describes similar forms in the blood of small-pox patients and in that of vaccinated calves and children during the fever stage. He spoke of them as ameboid, one-fourth to one-half the size of an erythrocyte. They contained nuclei, were ameboid, often possessed flagella, and swam free in the blood. He believed that these invaded the cells, and there developed into a second stage, the *Monocystis epithelialis*, whose contents divided into a number of spores, but the transition from these spores into the ameboid form was not worked out.

Of late years the most conspicuous articles have been by Dombrowski,² Funck,³ and Ishigami.⁴ Each of them claims to have found an organism with a more or less definite life cycle, and to have been able to reproduce the disease by inoculation of this organism. They all agree in placing it among the Sporozoa, but the resemblance ends there. Dombrowski finds that the vesicle fluid, with a magnification of 730 diameters, shows a "pure culture" of microbes, fine, dark, round, or elongated, most of them with a fine light border. They are always in lively motion, pendulum-like in character. Later stages show actual locomotion, perhaps

¹ Loc. cit.

² Zeitsch. f. klin. Med., xlv, 1.

³ Deutsch. med. Woch., 1901, No. 9. Cb. Bact., xxix, 921.

⁴ Cb. Bact., xxxiii, 794.

due to cilia. A little later, other transparent forms appear, two to three times the size of the foregoing, with indistinct outlines. These usually have four dark, central granules, actively motile, some granules being also free in the fluid. With the invasion of leucocytes, these small bodies decrease, and larger, regular shaped bodies are seen, more or less spherical, yellowish, with wavy contour and narrow white border. They are usually found in the protoplasm of the leucocytes, but are also seen separate. Besides these are diplococcoid forms resembling those of Pfeiffer. On stained slides these bodies are not seen, but unstained spaces may be noted in the leucocytes and in the blood. Bodies may be seen free, or in the erythrocytes or the leucocytes, similar to the smaller forms described in both light and severe cases. On these Dombrowski does not lay much stress. In his cultural experiments he uses plain agar, and examines the slant after some time in the incubator. He claims that a smear from the agar, examined in sterile water, shows similar bodies for a long time, and that smears made from the blood on agar slants give similar results. The surface of the medium, however, has no visible growth. The article is accompanied by a drawing of the forms seen, giving a very definite idea as to their appearance. He considers the various pictures to be parts of the life cycle of an organism which increases by sprouting. The ultimate details are not quite satisfactory, and it is hard to follow the different stages. No animal inoculations were attempted.

Funck has described an organism which he calls *Sporidium vaccinale*, and which has two chief forms — cysts filled with spores, and these same spores free in the fluid. The round cysts are about twenty-five mikrons in diameter, but may be as large as thirty-five, and show a membrane, sometimes apparently with a double contour. The spores are one to three mikrons in size, refractile, with ameboid movements. The author claims positive inoculation results, considering that by allowing the vaccine material, after thorough mixing with bouillon, to settle, and then skimming off the top, he thus procures a pure culture. To us this method seems

a little uncertain. He finds similar pictures in vaccine material, and in the contents of variola pustules. Other conclusions as to immunization by means of injections of the Sporidium are based on the above method of obtaining a pure culture, and are, of course, subject to the same objections. The plates accompanying the article are somewhat diagrammatic, but we have been able to recognize the majority of the forms noted.

Ishigami's work is a very extensive one. He seems to have had great facilities for obtaining material, and took up the problem from various points of view. He examined fresh material, tissues hardened in different ways, and claimed to have cultivated the organisms by a process of his own. The same author had previously isolated a blastomyces which he considered for a time to be the cause of the disease, but which he has now given up in favor of the present protozoon. Details as to appearances found in sections will not be taken up here, as they do not relate directly to the subject in hand. He places his organism among the Sporozoa, and describes a variety of forms which he thinks to be parts in the life cycle. In brief, he notes three stages, the first consisting of small, greenish refractile bodies of different sizes, some of them similar to Dombrowski's, others different. These progress in their development, showing well-marked ameboid movement at one stage, which he considers the growing stage, after which they form large sporocysts, each containing many spores. This process of development he sees in all ages of the material examined, and even in old dried crusts which have been treated with one per cent carbolic acid, and placed on the warm stage. These organisms he claims to have grown in a culture medium prepared on a basis of lymph obtained from a calf inoculated in the ear vein with fresh vaccine. His successful inoculations of "pure cultures" seem accordingly open to the same objections as Funck's, for, if the medium is the manifestation of inoculation with the vaccine material, it is not improbable that it contains some of the infectious agent. The description of the process is not very clear or

full in the translation, so that it is possible that we do not thoroughly understand it.

The scope of this article does not necessitate the analysis of all the other articles dealing with vaccine and variola bodies, and only such as bear directly on the work in this present series of experiments will be referred to.

Technic.—In order to obtain the material as free as possible from contaminations, the skin was cleansed with alcohol and the top of the lesion removed with a sterile point. This was found to be sufficient, if carefully done, to exclude the bacteria on the skin surface, and, furthermore, left no possibility of the presence of antiseptics to interfere with the subsequent development of any organisms present in the actual lesion. Sterile capillary pipettes were drawn from glass tubes, and made about the same diameter as the vaccine tubes in common use. With these the contents of the vesicle and pustules were drawn up, and, if the material was not to be used at once, the ends were sealed and the tubes preserved for future reference. It was found that there was no appreciable change after a period of several months in the ice-chest.

This material was examined in a variety of ways. For the most part it was analyzed in the hanging drop, on the warm stage, either stained or unstained. The stains used were osmic acid and Sudan III. for the demonstration of fat, and methylene blue for the demonstration of structure in the forms seen. The stain, when possible, was made up in normal saline solution, and, in any case, was added at the margin of the drop in such small quantities as to give only a very faint tinge to the fluid.

Dried smears were examined with osmic acid and Sudan III., some after fixation with formalin, others after alcohol and ether.

Some were stained with eosin and methylene blue, others with thionin, some with hematoxylin and eosin. The results of the examination of the fresh material will be taken up first, as most of our work was done with this.

In the first place, exhaustive examination under the oil immersion, at a magnification of 1,000, on a warm stage kept at 37.5 degrees Centigrade, never showed us any forms which we could bring ourselves to believe had any motion of their own, whether ameboid, ciliary, or due to flagella. All suspicious bodies were watched over periods of several hours, and drawn at intervals. At times there seemed to be definite change of shape in some of these, but protracted watching invariably showed a return to the original form at intervals, until we came to the conclusion that we were dealing with irregular débris which was turning slowly in the convection currents of the hanging drop. These observations were carried on over a period of several months, and we are convinced that persons who claim to have seen ameboid motion in the forms observed in the vesicles and pustules were either laboring under a misconception, or were dealing with something introduced from outside. The majority of the bodies seen by other observers, as above noted, were also seen by us. As many of the articles contained no diagrams, it was sometimes difficult to be sure, and some of the forms we never saw. The one upon which practically all observers agree was found in every case. We refer to the diplococcoid bodies described by Pfeiffer and emphasized by Dombrowski. These are seen in the leucocytes, in pairs, singly, or in groups, and in many cases appear to have a clear area about them, suggesting a capsule. This area, however, could not be stained with the ordinary capsule stains. These bodies were also seen free in the fluid, usually single or in pairs, and neither in the leucocytes nor free could they be stained by bacterial stains. When a mount is left in the warm chamber for two or more days, there is a well-marked increase in the number of these bodies. The cell, which at first shows only one or two, becomes sometimes quite filled with them, giving an appearance very similar to a cyst filled with spores. The nucleus becomes obscured, and sometimes quite invisible. In the majority of cases it may be seen on the addition of acetic acid. Some of the bodies become very large, oval or pear-shaped, and seem in places to have a double contour. Free in the fluid may be

seen some as large as the nucleus of a polymorphonuclear neutrophyl. These answer very well to the description of some of Ishigami's bodies in the intermediate stages. Some of the bodies resembled torulæ quite markedly. The color of these forms was distinctly greenish, and they were very refractile. Sometimes the diplococcoid bodies were scarcely greenish at all on the first day, but after twenty-four hours on the warm stage they became markedly so. The larger bodies were practically always greenish and refractile. Careful watching showed no change of form in the course of an hour or so.

Addition of a small drop of osmic acid at the border of the drop gave rise to a distinct reaction. This rarely took place at once, but became plain after a few hours. The granules became black around the borders, the center remaining clear at first, but gradually becoming black. This reaction was very suggestive, and the process was continued. Dried smears were treated with ether and stained with osmic acid, with no results. It is only fair in this connection to say that it is quite difficult to find these bodies on dried smears, even without the ether treatment. For this reason, and as an additional check, we made use of Sudan III. in seventy per cent alcohol. This was added in the same way and was very simple and satisfactory. The process of staining could be readily watched under the microscope, and was very sharply marked. The diplococcoid and other bodies noted were at first greenish, but in a few seconds began to take on a pink tinge, becoming darker and darker, until they were distinctly and deeply red, the outlines being remarkably sharp and clear cut. *All the bodies which answered in any way to the parasites described by the various authors took the Sudan*, while the cell débris and the nuclear fragments were not stained, but could be counterstained by the use of appropriate dyes.

In dried smears hardened with formalin we were able to obtain similar results, although the picture was somewhat distorted, but in smears fixed in alcohol and ether the results were uniformly negative. All these reactions point

directly to the origin of these "parasites" in fatty degeneration of cells. The forms include the sporozoites, growing forms and cyst forms of Ishigami, Dombrowski's diplococcoid and larger bodies, and practically all of Funck's forms. Podwyssowski and Mankowski¹ had already noted that Funck's bodies could be nothing but fat. Some of Ishigami's larger bodies, described as thirty-five mikrons in size, were not seen, our largest being only twelve to fifteen mikrons in size. These we interpreted as epithelial cells with fatty degeneration, and it seems probable that the large forms of Ishigami may have been aggregations of such cells.

Besides this Sudan staining material, there is in the contents of the vesicles and pustules a vast quantity of matter, most of it without form and void, much of it in such a condition that it is hard to say absolutely definitely just what it is. We have, however, seen in this mass of *débris* nothing which by staining methods, incubation, or general appearance we could bring ourselves to believe to be parasitic. Some bits can be identified as nuclear fragments, others as fragments of the cell bodies of leucocytes or epithelial cells, and some we could in no way identify. It is known that the contents of such vesicles and pustules is capable of reproducing the disease, and, therefore, there must be among this material some form of the infectious agent, but with the means at our disposal we were unable to come to any conclusions as to its form or appearance.

Blood Examinations. — Reed,² in his studies of the blood in variola and vaccinia, found, in a certain number of cases, small ameboid bodies in the blood in the first few days of the disease, both in man and monkeys. These he found with some uniformity, but found them also in one case in a person not suffering from the disease. He did not come to any absolute conclusion, but promised further work on the subject. Such work was prevented by his untimely death. Partly in view of this note, and partly on general principles,

¹ Podwyssowski and Mankowski. *Deutsch. med. Woch.*, xxvii, 261.

² Reed. *Journ. Exp. Med.*, ii, 515.

we made routine examinations of the blood in variola for some time, taking it from the ear at different stages of the disease and in cases of varying severity. The types ranged from one purpuric case through grades of disease down to varioloid, blood being examined from visitors to the hospital and from the men in the laboratory as a check on the results.

The mononucleosis noted by several observers, but best by Courmont and Montagard¹ and Ferguson,² was a conspicuous feature. No differential counts were made, but it was readily noticed both in the fresh blood and in the stained smears. The increase seemed to be chiefly in the large mononuclear cells. No special change was noted in the relations of the other types, but in the absence of blood counts no definite statements could be made. In the stained specimens, especially in advanced and severe cases, the polychromatophilia was a very marked feature, with the eosin-methylene blue stain. A number of preparations were made by pricking the skin through small drops of eosin, methylene blue, etc., in normal saline solution, in the hope of staining some bodies of interest, but beyond giving a very good picture of the leucocytes the attempt gave no results.

The usual appearances of blood were seen. There was a variable amount of hemaconium, besides the small granules supposed by hematologists to be extruded from the leucocytes, and found in normal blood in the same amount. There were occasionally somewhat larger bodies, pale and regular, staining faintly with eosin, which we supposed to be portions of red blood corpuscles, as they reacted in the same way to reagents, and disappeared under the same conditions. *In no case were any bodies found which in any way suggested ameboid forms.*

Animal experiments. — In connection with the above work a series of experiments on animals was undertaken, in the endeavor to induce development of the organisms which we believed to be present into some form in which we could recognize them.

¹ Compt. Rendus Soc. de Biologie, June 22-30, 1900.

² Journ. Path. and Bact., May, 1903.

Collodion sacs were prepared according to the method of Harris, and placed in the peritoneal cavities of rabbits. The sacs were filled with different sterile culture materials, including bouillon, gelatin, human and bovine blood serum. They were kept in the peritoneal cavity for variable periods, a check being made by means of the introduction of an uninoculated capsule. The inoculations were made with fresh material from vesicles and pustules of different ages, before the invasion of bacteria. Some inoculations were also made with blood from patients at different stages of the disease. A portion of the material inoculated was reserved and examined as a check on the later findings in the sacs from the animals. Cultures were also taken from the fluid before and after passage through the animal.

These experiments may be very briefly summed up as invariably negative. In no case were we able to find anything in the sacs from the animals different from the check made before. The cultures were also negative. The media remained unclouded, and exhaustive examination showed nothing we could claim as parasites.

The use of the anterior chamber of the eye in the study of the tubercle, and the well-known value of the cornea of the rabbit in the study of the vaccine bodies, led us to attempt the inoculation of the aqueous humor with variolous material.

The animal was placed under chloral anesthesia, and the cornea was additionally treated with cocaine. A special pipette was prepared for the inoculation. A hard glass tube was drawn out into a very fine point, and the large end arranged for a rubber bulb. After the introduction of a cotton plug half way down the tube, the whole was sterilized and the bulb fitted on. By means of the bulb, the variolous material, just taken from a vesicle or pustule and mixed with a small amount of sterile salt solution, was drawn up into the capillary end. The eye was flushed with sterile salt solution, and the tip of the pipette passed through the cornea into the anterior chamber, just outside the border of the iris. The aqueous humor at once mounted into the

tube, giving a thorough mixture of the two fluids, and was then gently driven by the bulb into the chamber. After holding the point in place for a few seconds, it could be withdrawn without appreciable loss of fluid. In itself the technic was successful, none of the eyes becoming infected, except for an acute conjunctivitis in one case, which cleared up at once under simple irrigations. The animals were killed at different times after inoculation, and the aqueous humor examined in hanging drop and by culture. Results were uniformly negative, and the method was finally abandoned.

Rabbits were inoculated in the ear vein with the blood of variola patients, but in the very severe cases the blood contained large numbers of streptococci, and in the lighter cases no results followed.

The methods suggested by Ishigami for obtaining culture material were tried on one calf, but no eruption followed in spite of repeated attempts, all of them, however, on the same animal, and, therefore, not conclusive as to the value of the procedure.

The undoubted fact that the contents of the vesicles and pustules is infectious, and must, therefore, have within it some form of the organism to which the disease is due; the evidence that this organism is of such a size as to be held back by the pores of a filter which does not restrain organisms we are able to see; and the fact that, in spite of protracted endeavors, we have been unable to see anything in this material which we could conscientiously say to be parasitic, leads us to the conclusion that the organism must be present in some form which, at least by the methods we had at our disposal, is not to be differentiated from the cellular debris which is so conspicuous in the field. This at once suggests looking farther back, and it seems to us that our work, in so far as it leads to this suggestion, is confirmatory of the recent paper of Councilman,¹ who finds his organisms

¹Councilman, Magrath, Brinckerhoff. Preliminary report. This Journal, Vol. ix, p. 372.

in recognizable form only in the very early stages of the variolous lesion. According to his ideas, all that there is in the vesicles and pustules, free in the fluid, is the spore stage, and inasmuch as these spores are said to be only one to two mikrons in size, they would be almost impossible to distinguish from the debris without some special stain.

The conclusions which we have reached as a result of the work noted in this paper, and review of the literature bearing upon it, are as follows:

- I. Variola is not due to a sub-microscopic organism.
- II. The organisms described by Dombrowski, Ishagami, and by Funck are not organisms, but various forms of cell-degeneration.
- III. The organism, or some form of it, is certainly present in the contents of the vesicles and pustules, but in some form in which we were unable to distinguish it from the cellular debris.

Pathological findings. — In the series of autopsies, forty in all, performed at the Detention Hospital, and in five other autopsies on variola cases, performed at various times in the last three years, besides the cultures elsewhere referred to, portions were preserved and hardened in Zenker's and in Orth's fluids from the heart, lungs, liver, spleen, pancreas, kidneys, adrenals, stomach, intestines, and skin. In cases where lesions were apparent pieces were also taken from the testicles, bladder, uterus, ovaries, larynx, trachea, and esophagus. Sections of these were cut, for the most part by the paraffine method, and studied with various stains. For the sections from the organs, where there were no variola lesions, hematoxylin and eosin were used as a routine. Sections from the skin, the larynx, and trachea, the esophagus, and anywhere lesions resembling those of variola were seen, were stained by Mallory's iron hematoxylin method and counter-stained with aurantia. Sections were also stained with Weigert's fibrin stain, and with eosin and methylene blue, to demonstrate the presence of bacteria. The series included

three cases of purpura variolosa, nine cases of secondary hemorrhagic type, twenty-seven cases of confluent variola, four of discrete, and two cases of varioloid, the latter dying of arterio-sclerosis.

The general pathological lesions will be summed up briefly, and the more important taken up separately. Cultural results are taken up in detail in another paper¹ and will not be discussed here.

Leaving out for the present the tissues which showed definite variolous lesions or pocks, we can summarize as follows:

In general, all the organs showed a constant and usually well-marked congestion, which in the hemorrhagic cases tended towards definite hemorrhages into the tissue.

In all organs susceptible to that change there was more or less well-marked cloudy swelling, extreme in many of the cases.

Taking up the organs one by one, the heart showed fibroid change in six cases, all of these showing well-marked arterio-sclerosis. In five cases there was thickening of the aortic and mitral valves, four of these associated with hypertrophy in moderate degree, and two of them sufficiently advanced to give rise to clinical symptoms.

The liver showed more or less fatty degeneration in twenty-three cases, the change in most of them being more distinct at the borders of the lobules, the centers and the central veins presenting a picture of marked passive congestion. Increase of fibrous tissue was noted in four cases, and deposition of bile pigment in the liver cells in two. In nearly every section there were numbers of liver cells in the branches of the portal vein, but no such cells were found in the lungs.

The spleen showed hemorrhage into the pulp in six cases, which includes the three hemorrhagic ones. In twenty-one cases it was larger than usual, and in three smaller, and the consistency was softer than normal in one-third of the cases, harder than normal in one-third, and normal in one-third. In general, the Malpighian bodies were very conspicuous, being less so than usual in only twelve instances. A marked feature in nearly all the cases was the presence of numbers of

¹ *Streptococcus pyogenes* in variola. This number, p. 180.

phagocytic cells in the pulp, containing erythrocytes as well as debris.

The pancreas showed nothing of especial interest, either macroscopically or microscopically.

The kidney showed chronic interstitial nephritis in marked degree in four, slight in two cases, and chronic diffuse nephritis in three to a large extent, less in two others. In one case there was an acute interstitial nephritis, the exudate containing plasma cells and eosinophiles, and a few polymorphonuclears. In two of the three purpuric cases there was marked hemorrhage just beneath the epithelium of the pelvis. The capsule was adherent only in the four marked interstitial cases.

The adrenals were cystic in six cases, and often showed well-marked fatty degeneration of the cells of the medulla. There were no hemorrhages into the tissue or the cysts.

In eight out of twenty-seven males the testicles showed well-marked necrosis. This was of the type usually described, with destruction of the tubules and infiltration of the intertubular tissue. Special stains for bacteria showed them in many of the vessels, and in places scattered through the tissue. Some of the smaller arteries contained thrombi, in which streptococci were seen.

No cases of necrosis of the ovaries or any lesion which we could attribute to variola were noted. In one case, not a hemorrhagic one, there was definite hemorrhage into the ovarian stroma. In six cases associated with arterio-sclerosis there was marked fibrous tissue increase.

The tubes showed no important changes, except that in one instance there was a hematosalpinx with a twisted pedicle, and chronic adhesions in one or two others.

The lungs showed congestion and edema in all cases, and frequently emphysema. In over thirty cases there was well-marked bronchitis. In those in which death occurred comparatively early, there were usually seen on the mucous membrane of the bronchi, in some cases even in those no greater in diameter than a slate pencil, distinct round or oval areas, more or less sharply defined, with a red areola, and

resembling exactly the areas noted in the larynx and trachea, of which, indeed, they seem to be the continuation. Histologically, also, they resembled these tracheal lesions. The involvement of the deep respiratory tract seems to be a very early one, for in many cases the skin lesions were far less advanced than those in the trachea.

It is partly on this seemingly early injury to the bronchi that we base one of the conclusions in another paper, that the portal of entry for streptococci is probably the lungs. In cases which came to autopsy later in the disease, the bronchitis for the most part was a general purulent type, and no distinct ulcers could be noted.

Broncho-pneumonia, thought by many to be one of the important causes of death, was in our series not of great significance. In ten cases there was well-marked broncho-pneumonia, in seven a few small areas, and in no case was so large a proportion of the lung involved as to suggest the lesion as a cause of death. Lobar pneumonia was not observed. One case showed infarction, apparently due to pyogenic thrombi, in which streptococci were found. Chronic tuberculosis was found in four cases.

The larynx and trachea were involved in thirty-three cases, in varying degree. In the majority of the larynges examined, distinct ulcerations were noted. In the earlier stages of the disease the ulcers were sharply cut and definitely like the pocks on the skin, with such differences as would of necessity occur from the difference in the tissues. In more extensive and later involvement the entire surface of the organ was covered with a foul greenish exudate, quite adherent, and showing marked loss of substance beneath when removed. The trachea was, as a usual thing, not so extensively involved as the larynx, and the lesions were more often separate and typical. As noted above, these separate ulcers were found in many cases as far down as the smaller bronchi, and were usually more advanced proportionally than those on the skin.

The serous cavities were not much affected. In the hemorrhagic cases there were small petechiæ in their walls, but in none was there distinct hemorrhage into the cavity. In only

three cases, and these the most extensive of the broncho-pneumonias, was there an acute purulent or fibrino-purulent pleurisy. In three cases there was effusion of clear serum in amount over one hundred cubic centimeters, and in quite a number there was a slight excess. Chronic apical and posterior adhesions were noted in twenty cases. The pericardium showed serous effusion over one hundred cubic centimeters in seven cases, and slight excess of serum in a number more. The peritoneum showed no changes of importance besides the petechiæ in the hemorrhagic cases.

In the first twenty-seven cases, and in the five outside, the esophagus showed no lesions. In the last thirteen there were five with well-marked and definite pocks similar to those seen in the larynx. The stomach and intestines showed marked congestion and occasional hemorrhages into the mucosa, but no pocks or other lesions.

The brain and spinal cord were uniformly congested, and in the cases of marked arterio-sclerosis showed more or less extensive thickening of the circle of Willis.

In conclusion, the lesions found at autopsy are simply those of any acute infectious disease. The epithelium of the internal organs, with the exception of such epithelium as is a direct continuation of the skin surface, does not seem to be a place selected by the organism of variola for its classical manifestations, and in our series, with the exception of the respiratory tract, it was rare to find pocks. The anus and rectum were not affected in any case, as far as we could see, and the urethra was involved only in a very few. Whether the special staining methods now being used in the skin lesions will bring forth anything new in sections of the organs is a question for the future to decide.

The special lesions showed nothing unusual with the ordinary stains. In the stages previous to the rupture of the pock no bacteria were seen, agreeing with the cultural work, and nothing in the way of parasites, more than the appearances described by various observers. Sections from lesions of appropriate age are now being examined in the light of Councilman's paper, but their description will not be here discussed, as our work is so far not conclusive.

STREPTOCOCCUS PYOGENES IN VARIOLA.¹

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Since first bacteria began to be identified with disease there has been a series of attempts to prove the bacterial origin of variola. Many bacteria have been described in this connection, some of them new, others old and well-known forms. Careful examination of the reports of the observers leads to but one possible conclusion, namely, that the final proof, the successful transmission of the disease by means of a pure culture, has never been successfully offered.

Leaving aside the special organisms, *Streptococcus pyogenes* has probably received more attention than any other. This is not strange in view of its almost universal presence in variola, and its apparent relation to the symptoms and sequelæ of the disease.

For this reason it seemed important to us to make a special study of the streptococci found in the autopsy and other material collected in the Cleveland variola epidemic of 1902-3.

The work fell naturally under several heads, which will be dealt with separately.

I. Frequency of streptococci and their localization in the body.—During our period of service there were forty autopsies, at each of which cultures were made by the plate method from the heart's blood, the lungs, the liver, the spleen, the kidneys, and in all cases where it was indicated, from the pleura, the pericardium, the peritoneum, the bladder, and the testicle.

Owing to the location of the morgue, it was necessary to perform the autopsies at night, so that there was frequently an interval of twelve to fifteen hours between the death and the post-mortem. In consideration of this, it was our practice

¹ Received for publication, July 29, 1903.

to take a blood-culture from the heart under aseptic precautions, as soon as possible after the body was moved from the ward. We were able to do this usually within an hour, almost always within two hours. As the majority of the cases died in cold weather, and the morgue was unheated, this gave a fair idea of the bacterial content of the circulating blood.

The cultural results in these autopsies may be summarized as follows: In thirty-eight cases streptococci were found distributed throughout the body in greater or less numbers, for the most part alone, but in some cases associated with the ordinary pyogenic staphylococci. In two cases there were no streptococci found. One of these was a man dying of arterio-sclerosis after recovery from a light attack of varioloid, and the other a baby dying the day after admission, with the purpuric form. It does not seem worth while to go into great detail concerning the number and type of the cases in which streptococci were found alone, and those in which they were in association with staphylococci, further than to note that *S. p. aureus* was found in about one-third the cases, without any special reference to their severity. Streptococci were, therefore, present in ninety-five per cent of all cases.

The exact proportion in the various types may be found in Table I.

TABLE I. — AUTOPSY CASES.

	Streptococci present.	Streptococci absent.	Streptococci in blood and most organs.	Streptococci in blood and one organ.	Streptococci with <i>S. p. aureus</i> .	Streptococci with <i>S. p. albus</i> .
Purpuric ...	2	1	1	1	
2d hem....	9	5	2	2	2
Confluent ..	24	12	11	11	1
Discrete....	1	1
Varioloid...	1	1	1			
Dessicating.	1	1			
Totals ...	38	2	20	13	14	4

In connection with this work, cultures were made from the blood during life to ascertain the proportion of cases showing streptococci, and the time of their invasion. The literature on this subject is meager. Arnaud¹ reports two positive cultures in hemorrhagic cases, and Ewing² negative results in ten varied cases.

The technic was that usually employed in blood culture work, ten cubic centimeters being taken from the arm vein under aseptic precautions, and divided between two flasks of bouillon, each containing one hundred and fifty cubic centimeters. Unfortunately, in some of the cases in which we were especially anxious to obtain cultures the eruption was so extensive and the skin so tender that the attempt had to be abandoned.

The cases will be taken up first according to the type of variola, and later summarized. Cultures were taken before death in thirteen cases, of which eight came to autopsy, and these latter will be considered first.

Purpura variolosa — 1 case.	1 hour before death,	Str. pyogenes.
zday hemorrhagic — 2 cases. —	I. 24 hrs. before death,	"
	($\frac{1}{2}$ hr. after death),	"
	II. 24 hrs. before death,	S. p. albus.
Confluent — 5 cases. —	I. 3 days before death,	Str. pyogenes.
	(2 hrs. after death),	"
	II. 7 days before death,	Negative.
	4 " " "	Str. pyogenes.
	1 " " "	"
	III. 6 " " "	Negative.
	3 " " "	Str. pyogenes.
	($\frac{1}{2}$ hr. after death),	"
	IV. 2 hrs. before death,	"
		S. p. aureus.
	V. 7 days before death,	Str. pyogenes.
		S. p. aureus.

The remainder of the thirteen cases did not die, and may be grouped as follows:

Confluent — 3 cases. —	I. Severe. 5th day of eruption,	Negative.
	II. Moderate. 7th day of eruption,	Str. pyogenes.

¹ Rev. de Med., 1900, p. 303.

² Proc. N.Y. Path. Soc., May, 1902.

	III. Moderate. 4th day of eruption,	Negative.
	8th " " "	"
Varioloid — 1 case. —	I. Ordinary. 5th " "	"
Convalescent confluent — 1 case. —	18th " "	"

In seven cases, five of them very severe, in which it had not been possible to obtain blood before death, cultures were made immediately after, within one hour.

Purpuric — 3 cases. — I. Negative.
II. Streptococcus pyogenes.

III. S. p. albus (?).

Confluent — 2 cases. — I. Streptococcus pyogenes.
II. " "

Staphylococcus citreus.

Varioloid — Arterio-sclerotic death.

2 cases. — I. S. p. albus (?).

II. " " "

In the three cases marked S. p. albus (?) there was a late development of staphylococci, indicating a very few colonies, which may have been contaminations.

In five other cases, for one reason or other, cultures were not taken until three to six hours after death.

Secondary hemorrhagic — 3 cases. — I. S. p. aureus. Autopsy showed also streptococci.

II. Str. pyogenes.

III. " "

Confluent — Late — 2 cases. —

I. " "

II. Bacillus coli.

Dessicating —

I. " "

Omitting these last on account of the interval elapsing, it seems fair to classify the others together, under the head of fatal and non-fatal cases.

I. Fatal cases.		Streptococci.	No streptococci.
Purpuric. — 4 cases	2 or	50 per cent	2 or 50 per cent
Secondary hemorrhagic. — 2 cases,	1 " 50 " "		1 " 50 " "
Confluent. — 7 cases	7 " 100 " "		
Varioloid. — 2 cases	2 " 100 " "		
II. Non-fatal cases.			
Confluent. — 8 cases	1 " 33½ " "		2 " 66½ " "
Varioloid. — 1 case			1 " 100 " "
Convalescent. — 1 case			1 " 100 " "

This makes a total of twenty cases, with streptococci present before or just after death in eleven cases, or fifty-five per cent. Omitting the varioloids and convalescent, and considering only the more serious cases, a total of sixteen, with streptococci in eleven, or sixty-nine per cent.

As to the time of invasion of the blood, the number of experiments which could be carried over a series of some days' duration is too small to enable us to speak dogmatically, but the absence in the less severe cases even during the full pustular stage, the absence in varioloid, and the absence in the local lesions, to be referred to later, seem to justify our conclusion that *Streptococcus pyogenes* has nothing to do with the change from the vesicular to the pustular stage.

The absence in those cases which die early, especially the purpuric and secondary hemorrhagic, in which streptococci were not found regularly, in spite of the use of large amounts of blood, would seem also to indicate that in these types the severity of the disease is dependent rather on the primary variolic infection than on the secondary streptococcic infection.

Cultures were made from a number of the local lesions at different stages in their development, and also from the secondary abscesses and other late infections.

The results from the local lesions were so uniform that detail is unnecessary. The skin in the first cases examined was treated with alcohol, followed by corrosive sublimate, which was again washed off with alcohol. The top of the lesion was then removed with a sterile needle, and the culture taken from the bottom layer. Cultures were made on various media, on which the streptococci isolated from other sources were found to grow readily, and check coverslips were made in each case. The uniformity of the negative results led us to abandon the corrosive sublimate in the fear that sufficient might be carried over into the culture medium to inhibit growth. There was, however, no change in the results.

In thirty cultures made in this way from the typical variola lesions at all the various stages, from the beginning vesicle to the full development of the ripe pustule, cultures and coverslips were negative in all but four. These fell on the eighth, ninth, and tenth days, two of them being on the tenth day. The positive culture on the eighth day showed only *S. epidermidis albus*, and one of the tenth-day cultures showed only *S. p. aureus*. Of the two cultures which showed the presence of streptococci, the one on the ninth day was from a large bleb on the wrist, over a centimeter in diameter, and containing cloudy serum. In this *S. pyogenes* was found alone. The positive culture on the tenth day was taken from a large single pustule, three-fourths centimeter in diameter, and showed both *S. pyogenes* and *S. p. aureus*.

Twelve cultures taken from secondary abscesses in various parts of the body showed streptococci alone in five, and in combination with pyogenic staphylococci in six. In one abscess the coverslip showed only staphylococci, and the culture grew out as *S. p. albus*. Three cultures from purulent conjunctivitis showed streptococci in combination with pyogenic staphylococci.

Cultures made from the lesions some hours after death, as a rule, showed the presence of streptococci, but as there was evidently a thorough distribution of organisms in all parts of the body, as evidenced by the autopsy cultures, we have confined our attention to the cultures taken when any post-mortem or agonal distribution can be excluded. The negative results in cultures agree with those of most observers, and the report of Ewing,¹ in which he finds positive results in fifteen out of seventeen vesicles, is based on post-mortem work, and agrees with our cultures after death, as noted above.

Le Dantec² states that the streptococcus which he isolated in variola and considered as the etiological factor is in the lesions in the condition of an obligate anaerobe, a characteristic which it loses after a few generations of cultivation

¹ Loc. cit.

² Arch. de Med. Navale, 1895, p. 410.

without oxygen. Our anaerobic cultures were negative in the few cases in which we tried the method, and the uniform absence of organisms in the coverslips, except in those cases with positive cultural results, seems fairly conclusive evidence against such an unusual state of affairs.

II. Value of anti-streptococcus serum. — In view of the very favorable results said to result from the treatment of scarlet fever by anti-streptococcus serum and the similarity of the apparent relation of streptococci to both diseases, we tried serum in a series of cases, for two ends. We desired, in the first place, to find out whether the acute symptoms were due in any way to *Str. pyogenes*, and if so, whether the administration of anti-streptococcus serum would ameliorate them, and in the second place, whether we could induce an immunity against that organism such as to prevent the secondary infections due to it, infections which occurred in the majority of the cases.

As far as was possible, parallel cases were studied, the one being treated with serum, the other being left untreated, to ascertain whether there was any special difference to be noted.

Nine cases in all were treated in this series. Two were confluent, with bad prognosis from the beginning, the serum being given when the lethal exitus was in sight. Two were secondary hemorrhagic in type, one being in the same condition as the above cases, the other being taken earlier. Two were less extensive confluent cases with good prognosis. Three were simple discrete and confluent cases. In the four severe cases streptococci were found in the blood during life, the others being in a condition in which blood cultures were impracticable. In each case the temperature was taken before and after the administration of the serum. Anti-streptococcus serum will be denoted by AS.

CASE SUMMARY. — Case I. — Palmer. Male, 22. No vaccination. Adm. XII./12. Died XII./21. Eruption of severe and universal confluent type. Admitted on the 2d day of eruption. Temp. on admission, 99.8°. Steady rise to day of death.

XII./19. 9th day, 12 M. 40 cc. AS. T. one hour before, 103.6°;
after, 105.0°.

7 P.M. 40 cc. AS. T. one hour before, 104.0°;
after, 105.0°.

XII./20. 10th day, 12 M. 20 cc. AS. T. one hour before, 103.6°;
after, 105°.

No change for the better was seen, and the patient was very weak. The treatment was discontinued, and death took place the next morning. Streptococci had been demonstrated four days before death, and were found immediately after death in the blood and at autopsy in all the organs.

Case II. — Curry. Male, 30. No vaccination. Adm. XII./15. Died XII./21. The case was very similar to the above. Temp. on admission, 99.9°.

XII./19. 6th day, 12 M. 20 cc. AS. T. one hour before, 100.8°; one hour after, 102°.

7 P.M. 20 cc. AS. T. one hour before, 102.4°;
one hour after, 102.6°.

7th day, 8 A.M. 20 cc. AS. T. one hour before, 102.8°; one hour after, 102.4°.

8 P.M. 20 cc. AS. T. one hour before, 102.4°; one hour after, 102.6°.

There was no amelioration of the patient's condition, and death followed the next morning. Streptococci were found in the blood the third day before death and at autopsy in all the organs.

Case III. — Murphy. Male, 36. Vaccinated 30 years ago. Adm. XII./11. Died XII./17. This case was very similar in appearance to the other two on admission, but developed into a secondary hemorrhagic during the last two days of life. The temp. on admission was 105.0°, and varied between this and 102.4°.

XII./17. 1 P.M. 20 cc. AS. T. one hour before, 102.4°; one hour after, 103.8°.

8 P.M. 20 cc. AS. T. one hour before, 105.0°.

Died at 9 P.M. Streptococci were found everywhere. This was one of the cases in which no blood culture could be taken.

Case IV. — Morgan. Female, 22. No vaccination. Adm. XII./26. Died XII./31. Admitted on the first day of the eruption. Soon after the type became secondary hemorrhagic. Temp. on admission, 100.4°.

XII./27. 2d day, 3 P.M. 20 cc. AS. T. one hour before, 103.2°; one hour after, 102.4°.

28. 3d day, 3 P.M. 10 cc. AS. T. one hour before, 100.4°; one hour after, 101.2°.

29. 4th day, 3 P.M. 10 cc. AS. T. one hour before, 103.2°; one hour after, 103.4°.

30. 5th day, 3 P.M. 10 cc. AS. T. one hour before, 103.4°; one hour after, 103.0°.

The condition of the patient became steadily worse, and death occurred on the 31st. No blood culture had been taken, but the blood immediately after death and cultures at autopsy showed large numbers of streptococci.

The following cases were lighter in type, and were treated in the hope of preventing some of the secondary infections.

Case V.—Grout. Male, 30. Vaccinated in childhood. Adm. XII./18. Discharged I./6.

Admitted on 2d day of eruption. The case was of moderate severity, discrete, and confluent in type. Temp. varied from 97.8° to 103.8°.

- XII./19. 3d day, 10 A.M. 10 cc. AS. T. one hour before, 98.8°; one hour after, 100.2°.
20. 4th day, 4 P.M. 10 cc. AS. T. one hour before, 101.0°; one hour after, 101.0°.
21. 5th day, 4 P.M. 10 cc. AS. T. one hour before, 102.2°; one hour after 102.8°.
22. 6th day, 8 P.M. 10 cc. N. T. one hour before, 102.2°; one hour after, 102.0°.
23. 7th day, 8 P.M. 20 cc. AS. T. one hour before, 102.8°; one hour after, 103.8°.
25. 9th day, 8 P.M. 20 cc. AS. T. one hour before, 102.0°; one hour after, 100.6°.
26. 10th day, 8 P.M. 10 cc. AS. T. one hour before, 99.0°; one hour after, 99.4°.
27. 11th day, 8 P.M. 10 cc. AS. T. one hour before, 101.0°; one hour after, 102.4°.

This case ran a course similar to the untreated case watched. There appeared to be no shortening of the period before convalescence. Two or three days after cessation of treatment the patient developed what was apparently rheumatism, first in one shoulder, then in the other, and subsequently had a series of secondary boils to the usual degree consistent with the severity of his attack.

Case VI.—Shroeder. Male, 22. Vaccinated in childhood. Adm. XII./27. Disch. I./17.

Admitted on the second day of a light attack of discrete variola.

- XII./28. 3d day, 3 P.M. 20 cc. AS. T. one hour before, 100.2°; one hour after, 100°.
29. 4th day, 3 P.M. 10 cc. AS. T. one hour before, 100.2°; one hour after, 100.4°.
30. 5th day, 3 P.M. 10 cc. AS. T. one hour before, 101.0°; one hour after, 101.4°.
31. 6th day, 3 P.M. 10 cc. AS. T. one hour before, 101.2°; one hour after, 100.0°.

In this case the secondary infections were slight, and contained only staphylococci, but the parallel cases watched and untreated were also practically free.

Case VII. — Weisman. Male, 46. Vaccinated in childhood. Adm. XII./29. Disch. I./20.

Admitted on 2d day of eruption. Case was a moderate confluent one.

Three days after admission it was decided to try the serum. On examination of the case there was an appearance suggesting the possibility of abortion of the eruption, but we decided to give one treatment at any rate. Another case in the adjoining bed was in a similar condition, and was watched as a comparison.

I./1. 5th day, 3 P.M. 15 cc. AS. Temp. one hour before, 100.6°; one hour after, 101.0°.

The next day the eruption had changed in appearance and went on rapidly to abortion. No further treatment was given, and this case, as well as the parallel untreated one, went on to rapid convalescence, with only a few minor infections.

In view of the possibility, on which another paper¹ is based, that the invasion of *Str. pyogenes* might be due to a loss of the complement in the blood, and that accordingly the anti-streptococcus serum might have nothing with which to enter into combination, normal horse serum, containing the usual amount of complement, was obtained. This was used in two cases in combination with the anti-streptococcus serum. In case V. 10 cc. of normal was given one day, as the supply of anti-serum had given out, and the patient wished the treatment continued, but this was not followed up. In the next two cases, however, the two were given together throughout the time of treatment. Normal serum will be noted as N.

Case VIII. — Osika. Male, 30. Vaccinated as a child. Adm. I./3. Disch. ca. I./28.

Admitted on 2d day of eruption. Moderate confluent case. Temp. 101.4°.

I./5.	30 cc. AS.	T. before, 101.2°.	After, 101.2°.
7.	20 " " 20 cc. N.	" 99.6°.	" 101.2°.
8.	20 " " 20 " "		

Blood culture on I./7 negative. This patient received 60 cc. anti-serum and 40 cc. normal serum in five days. The case went on to cure and discharge uneventfully. The secondary infections were mild and unimportant.

Case IX. — Jackson. Male, 18. Vaccination in childhood. Adm. I./11. Disch. ca. II.

Admitted on 2d day of eruption. Simple discrete. Temp. never high.

I./12.	A.M. 20 cc. AS.	T. before, 99.6°.	After, 97.8°.
	P.M. 20 " " 20 cc. N.	" 101.4°.	" 99.8°.
13.	20 " "	" 102.2°.	" 102.0°.
14.	20 " "	" 101.6°.	" 101.6°.
16.	10 " " 10 " "	" 102.0°.	" 101.4°.

¹ Bactericidal action of blood serum in variola. This number, p. 196.

The total amount given was, therefore, 70 cc. of anti-serum and 50 cc. of normal serum. Three days after the cessation of treatment, the patient developed marked purulent conjunctivitis, with the presence of streptococci in the exudate. Later he developed the usual amount of secondary infections, from which streptococci were recovered.

Serum inoculations had to be given up at this point, because the visiting physician to the Detention Hospital wished to try the efficiency of intravenous formalin injections. The experiment was therefore not carried out to the extent we desired, but even with this small number the results were very uniform. In the usual course of events some patients were more free from infections than others, and in our series it so happened that the untreated cases watched as controls were so similar in their outcome that without a previous knowledge it would have been impossible to say which was which. One or two of the cases might have seemed to us to be improved, had it not been for the careful comparison with the parallel cases, and with other similar ones, at different times. The case that aborted after one treatment had, in our opinion, no change attributable to the serum. Not only the case used as control, but three or four others of similar type, aborted and went on to convalescence without any other than the usual treatment. The addition of the normal serum seemed not more valuable than the anti-serum alone, and together with the experiments in the other paper referred to, seemed to indicate some lack other than complement to explain the secondary infections with streptococci. Where there were streptococci in the blood before death, there was no apparent diminution, and cultures at autopsy showed the organisms everywhere in very large numbers.

The temperature changes after the administration of the serum were very variable, and seemed to us to bear relation rather to the amount of handling necessary in giving the dose, than to the action of the serum. The greatest rises were in large, heavy men, who required considerable effort to keep them in position.

In conclusion, treatment with anti-streptococcus serum in our hands had practically no effects for good or evil, either on

the general course of the disease, or on the secondary infections in which *Str. pyogenes* is found.

III. Identity of streptococci in variola. — In seeking an explanation for this total lack of effect, it seemed to us probable that the cause might be that we were dealing either with a variety or strain of streptococcus different from that used in the preparation of the serum, or even with a number of varieties. With this in mind, a number of cultures of streptococci from various lesions in our series were preserved, and compared in various ways. A list of the sources will not be out of place as indicating the range covered. Sixteen in all were used, labeled as follows:

1, Secondary abscess; 2, kidney, aut. 24; 3, blood culture 20 minutes after death, aut. 21; 4, blood culture, 4 days before death; 5, lung, aut. 23; 6, heart, aut. 27; 7, spleen, aut. 28; 8, eye, purulent conjunctivitis (Case IX. anti-serum cases); 9, lung, aut. 30; 10, kidney, aut. 30; 11, spleen, aut. 35; 12, intra-muscular abscess, aut. 36; 13, rabbit inoculated with blood from patient; 14, lung, aut. 38; 15, blood culture, 2 hours after death, no autopsy; 16, blood from heart, aut. 31.

As a comparison with these, streptococci from other sources were examined. These were numbered as follows:

17, streptococcus used in the preparation of the anti-serum used; 18, streptococcus from a case of articular rheumatism; 19, streptococcus from vaccine S.; 20, streptococcus from vaccine A.; 21, streptococcus from scarlatinal sore throat; 22, streptococcus from puerperal sepsis.

These twenty-two organisms were grown on the ordinary media, and showed certain constant differences which may be briefly summarized.

On agar slants of the different kinds of agar most of the colonies were fine, transparent, edges fairly regular, the substance of the colony showing finely granular under the low power. Even after many days' growth these colonies remained small. On the other hand, the cultures numbered 8, 15, 18, 20, showed much larger colonies, which were also less

transparent, more irregular in contour, and distinctly coarser in their granulations under the low power.

In milk the variation was perhaps the most marked. No change in three weeks, 8, 14, 19, 20; slight acid at the end of three weeks, 10; slight acid in forty-eight hours, no further change in three weeks, 1, 2, 3, 4, 6, 7, 9, 11, 12, 15, 16, 21, 22; slight acid in twenty-four hours, no further change in three weeks, 13, 17; acid after forty-eight hours; coagulated in ten days; no further change, 5, 18.

In gelatin there was no liquefaction in any case. The growths were fairly similar, some growing more rapidly than others. This was especially noticeable in 6, 7, 8, 9, 20. Numbers 4, 11, 18 grew more slowly than the average.

On potato there was no visible growth in any case in three weeks' examination. The organisms could, however, be recovered on sub-culture after a week.

Bouillon of two varieties was used, the one being the ordinary bouillon, prepared according to the specifications of the American Association of Public Health, but with a reaction of neutral to phenolphthalein; the other prepared from horse flesh, and reacting alkaline to phenolphthalein, as recommended by Aronson.¹

The majority of the cultures grew in the form of a sediment, or with small particles adhering to the sides of the tube, 1, 2, 3, 4, 7, 8, 9, 10, 12, 14, 16, 21, 22.

The rest showed a varying degree of cloudiness in the bouillon, with the presence of a more or less marked sediment, 5, 6, 11, 13, 15, 17, 18, 19, 20.

As a further test of the identity or non-identity of the cultures, the agglutination of the organisms when acted on by the anti-serum used in the treatment of cases was tested as far as possible. The technic used was that of Meyer, and, as in his cases, only those cultures which gave a distinct cloud in bouillon were used. The others were in an apparent condition of agglutination already; at least, so far as could be seen in the test-tube and with the microscope, and addition

¹ Berl. klin. Wochens., Oct. 20, 1902.

² Ibid., Oct. 6, 1902.

of the serum made no apparent difference. As noted above, nine of the cultures grew with a diffuse cloud, and were accordingly available for the experiment. Fortunately the organism from which the serum was prepared grew in this way.

Portions of the cultures were mixed with equal quantities of the anti-serum, and placed in the incubator at 37.5° Centigrade for five hours, so arranged that they could be inspected at intervals, without shaking them in any way. The experiment was repeated several times, with the same results. Number 17, the one from which the anti-serum was prepared, was affected at the end of the first hour, and in five hours the fluid above was clear, the growth being all sedimented to the bottom. The others showed no change in the first two hours, at the end of which No. 6 showed a slight sediment, somewhat more marked at the end of the five hours, but with a continued cloudiness of the fluid. At the end of four hours No. 5 showed some sedimentation, but the medium remained cloudy. The rest showed no change during the experiment. After twenty-four hours Nos. 5, 6, 11, 13, 15, 18 showed well-marked sedimentation, but the check tubes, consisting of another portion of the same culture unmixed with serum, and kept under the same conditions, showed similar sedimentation, except that there was a little more sediment, and the media above was a little clearer in the treated tubes of Nos. 5 and 6 than in the untreated.

So that of nine cultures tested against the anti-serum used in the treatment of cases, only one, and that the one used in the preparation of the serum, was agglutinated by it in any marked degree. In two other cases there was possibly a feeble reaction, but not conclusive.

These various and constant differences in the laboratory manifestations of the cultures at hand seem to us to justify the conclusion that we are dealing with several strains of streptococci, rather than with a single one, and the absence of effect of the anti-streptococcus serum, together with the fact that this serum agglutinated only the culture from which it was prepared, indicate that they were all different from this latter.

The results of the work done in this series of experiments agree markedly with Hektoen's valuable paper¹ on streptococci in scarlet fever. In fact, as far as we can see, these organisms bear much the same relations to both diseases, occurring not as the essential cause, but rather as secondary invaders. In this connection it seems reasonable to us to suggest the lungs as the probable portal of entry.

Streptococcus pyogenes is well known as a frequent, if not usual, inhabitant of the throat, even in healthy persons; sore throat, in the series of cases which we had the opportunity of studying, was practically universal; there was early involvement of the lungs in practically all cases, giving a point of least resistance; with these premises the conclusion that the streptococci found in the blood and organs entered by this route is a natural one. The evidence that we are probably dealing with several strains, rather than with a single one, adds weight to this opinion, as the organisms in the throats of a variety of people coming from different places are likely to be of different strains.

The pathogenicity of the organisms isolated was markedly different. Some of the strains killed rabbits in two or three days, while others were without effect. The observers had one or two infections from the work, all of which remained localized. Cultures which had been kept any length of time lost their pathogenicity to a marked degree.

It has accordingly seemed to us that this variety of organisms, or strains of organisms, the uncertainty of their presence in the blood, the certainty that in many severe cases they are not present in the circulation, unless the later stages of the eruption are reached, their absence in coverslips and cultures from the typical lesions up to and including the late pustular stages, indicate that, as suggested above, their presence is subsidiary, and even, so to speak, accidental.

The pustulation is due to the primary infection and not to the secondary invaders, as these are not found in that stage. It is, however, probable that the late streptococcus bacteriemia hastens death in many cases, and that the secondary abscesses

¹ Journ. Am. Med. Assoc., 1903, 685.

and other infections may in many cases delay convalescence, and even cause death by long-continued suppuration.

A brief summary of our conclusions is as follows :

I. Streptococci are present in all, or nearly all, severe cases of variola which prove fatal, and in many which recover. They may occur in the blood some days, or only a few hours, before death, and multiply very rapidly after death.

II. They bear no etiological relation to variola.

III. They bear no relation to the pustulation of the skin lesions.

IV. The probable portal of entry is the bronchial mucous membrane.

V. The streptococci found in the blood and the secondary infections are not identical, but vary markedly in certain constant characteristics.

VI. Probably because of the last conclusion, anti-streptococcus serum prepared from one variety of streptococcus is of no especial value in combating the symptoms and sequelæ of variola which are due to streptococci.

BACTERICIDAL ACTION OF THE BLOOD SERUM IN VARIOLA AND IN VARIOLOID.¹

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The presence of *Streptococcus pyogenes* in nearly one hundred per cent of the autopsies performed by us at the Detention Hospital in Cleveland in the winter of 1902-1903 led to the consideration of the possibility that this invasion might be due to a definite loss of the bactericidal power in the blood, especially against that organism.

Longcope's² work on the loss of complement in wasting diseases had given strong indications that such loss had a powerful influence on the secondary and often fatal infections in such diseases, and it seemed not improbable that some similar change in the blood might lead to this frequency of *Str. pyogenes*.

At the very outset we were met by a serious obstacle in the fact that streptococci will grow not only in serum from which the complement has been removed, but also in normal serum, and even in anti-streptococcus serum, specially prepared against it.

No method of direct test which might overcome this difficulty occurred to us, and we were accordingly forced to test merely for a general loss of complement, using organisms more easily killed than streptococci. For various reasons we selected the typhoid and colon bacilli.

The technic followed in this series of experiments was adapted from Longcope's work, with certain modifications. We thought it better to use smaller numbers of the bacteria, with the idea that a larger number might overwhelm a remnant of the complement and finer changes be thereby hidden.

¹ Received for publication July 29, 1903.

² Study of the bacteriolytic serum-complements in disease. Univ. of Penn. Medical Bulletin, November, 1902.

Blood was obtained from two sources — autopsies and living patients; and normal serum for comparison was obtained from healthy persons during life.

The blood was collected under aseptic precautions, allowed to coagulate, and the serum drawn off. In many of the cases the blood contained streptococci before it was drawn, and was accordingly filtered through a Pasteur filter before use.

Tubes were made as follows: I. Serum inoculated with typhoid. II. Serum inoculated with colon.

In addition checks were made as follows: III. Serum uninoculated as a check on its sterility. IV. Normal serum inoculated with the organisms under consideration. V. Bouillon inoculated with the organisms to make sure they were viable. As regards these last three, it is only necessary to say that experiments in which sterile serum could not be obtained have been omitted, and that the normal serum killed the organisms with uniform regularity. The growth in bouillon was always rapid, the plates being uncountable at the end of twenty-four hours.

In tubes I., II., III., one cubic centimeter of serum was used each time, and plates were made at once in four, eight, and twenty-four hours, and counted at the end of twenty-four hours from the time of inoculation. In all cases where the organisms inoculated were apparently present at the end of the twenty-four hours they were identified in the usual manner, the Widal test being used with the suspected typhoid.

The cases will be taken up according to the type of the disease, and a comparison of the series made later.

The first four cases died of variola of a very severe confluent type, in the later stages of pustulation, the blood being taken immediately after death before the performance of the autopsy.

In the records and tabulations which follow, the numeral I. refers to the serum inoculated with *B. typhus*, and the numeral II. to the serum inoculated with *B. coli*.

Case 1.—Male, white, 30; adm. XII./15; died XII./21; no vaccination. Died on the 7th day of eruption. Blood taken one-half hour after death. 80 cc. anti-streptococcus serum given in previous twenty-four hours.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	188	59	7	0
II.	1,105	455	361	Uncountable.

Case 2.—Female, white, 19; adm. I./13; died I./18; never vaccinated. Died on the 7th day of eruption. Blood taken one-half hour after death.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	153	5,135	8,328	Uncountable.
II.	505	8,320	Uncountable.	"

Case 3.—Male, white, 34; adm. I./27; died I./28; never vaccinated. Case well advanced on admission. Died about the 10th or 11th day.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	131	1,235	2,730	Uncountable.
II.	437	9,100	Uncountable.	"

Case 4.—Female, white, 22; adm. II./12; died II./21; never successfully vaccinated. Died about the 10th day. Blood taken one hour after death.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	210	103	150	8,905
II.	171	2,860	5,150	Uncountable.

The second group includes three cases of the secondary hemorrhagic type, dying on or before the sixth day. Only one of these was tried against colon.

Case 5.—Female, white, 30; adm. II./17; died II./20; never vaccinated. Died on 4th day. Blood taken six hours before death. Six months pregnant.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	75	0	0	0

Case 6.—Male, white, 39; adm. II./24; died II./28; never vaccinated. Died on 5th day. Blood taken two hours after death.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	57	0	0	0

Case 7.—Male, white, 23; adm. I./27; died II./1; never vaccinated. Died on 6th day. Blood taken one hour after death.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	119	0	0	0
II.	563	0	0	0

The next case is a baby, with a scanty, discrete eruption, who died on the 3d or 4th day of eruption, with a clinical diagnosis of broncho-pneumonia. No autopsy could be obtained.

Case 8.—Female, white, 6 months; adm. II./15; died II./16; never vaccinated. Died on 4th day. Blood taken two hours after death.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	130	0	0	0

The next case is another baby, dying on the day after admission with purpura variolosa. The child was much emaciated, and apparently in a chronic state of "malnutrition."

Case 9.—Female, white, 3; adm. I./3; died I./4; never vaccinated. Died on 3d day. Blood taken two hours after death.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	975	2,015	20,000	Uncountable.
Tube II.	755	9,651	25,000	"

The next two cases were neither of them considered as markedly serious, but developed a large number of serious secondary infections, from which streptococci were cultivated, and they died after all symptoms of variola had subsided.

Case 10.—Female, white, 20; adm. I./24; died II./16; never vaccinated.

The case was one of a moderate confluent type, but in the dessicating stage developed numerous secondary abscesses, both subcutaneous and intra-muscular. She was given five subcutaneous injections of formaldehyde, two of them 1/5000, three of them 1/3000, the amount in each case being 750–1000 cc. Blood taken one-half hour after death.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	127	274	2,304	20,000
II.	603	10,660	35,000	Uncountable.

Case 11.—Female, white, 43; adm. I./6; died II./7; never vaccinated.

The secondary infections developed were similar to those in Case 10. Blood taken one-half hour after death.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	950	1,690	4,225	4,875
II.	455	9,295	35,000	Uncountable.

The next succeeding case is one of a five months' baby, dying in the dessicating stage. The baby was nursing when taken ill, and inasmuch as its mother was also suffering from variola, was unable to obtain proper nourishment.

Case 12.—Male, white, 5 months; adm. I./8; died, II./5; never vaccinated. Died in late dessicating stage. Blood taken two hours after death.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	306	654	2,600	25,000
II.	400	6,315	12,000	35,000

The next case is one of discrete variola not expected to die. There was a clinical diagnosis of renal involvement, but no autopsy could be done on the case.

Case 13.—Male, white, 35; adm. II./17; died III./4; vaccinated in childhood. Died in dessicating stage. Blood taken 2 hours after death.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	131	0	0	0

The blood of five cases which did not come to autopsy, but were discharged cured, was also examined in this series. Three of these were confluent in type, one of them being of the abortive form.

Case 14.—Male, white, 32; adm. I./17; disch. II./16; vaccinated in childhood. Blood taken on 6th day of eruption. Culture showed streptococci.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	304	259	196	1,005
II.	591	2,145	20,000	Uncountable.

Case 15.—Male, white, 40; adm. I./3; disch. I./29; vaccinated in childhood. Blood taken on 5th day of eruption. Type abortive confluent. The blood was taken at the beginning of the abortive period. Blood culture negative.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	20	0	0	0

Case 16.—Male, white, 30; adm. I./3; disch. I./26; vaccinated in childhood. Blood taken in convalescence. Culture negative.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	351	693	1,398	5,605
II.	3,005	Uncountable.		

The next case was one of simple variola, of the discrete type; adm. I./17; disch. II./16; vaccination in the last few years. Blood taken in convalescence. Culture negative.

	At once.	4 hours.	8 hours.	24 hours.
Tube I.	49	0	0	0

The last case was a varioloid, vaccinated in the last few years. The blood was taken about the third day of the eruption; adm. I./27; disch. II./8.

The number of cases is not very large, and the amount of other work on hand did not admit of as much amplification of the experiments as we should have liked, but it seems to us that the cases are sufficiently characteristic to admit of some conclusions as to the presence or absence of the general bactericidal complement.

A summary of the whole series is as follows: Fatal cases, thirteen. In four cases of severe confluent variola, dying in the late pustular stage, the serum of three showed no hindrance to the growth of the organisms, which multiplied almost as rapidly as in the check bouillon. In the other case, the colon bacillus count fell from eleven hundred to three hundred and

fifty in eight hours, and then rose very much up to twenty-four hours; while the typhoid bacillus count fell steadily but slowly from one hundred and eighty-eight to seven in eight hours, and organisms were entirely absent in twenty-four hours. In three cases of the secondary hemorrhagic type, dying about the fifth or sixth day, the organisms were killed at the end of four hours. In one case of discrete variola, dying of broncho-pneumonia on the third or fourth day, the result was the same. In one case of purpura variolosa in an emaciated baby the organisms multiplied rapidly. In three cases dying late, in advanced dessication, two of them as a result of long-drawn-out infections with abscess formation, there was marked increase of the inoculated organisms, though not to the same extent as in the check bouillon. In one discrete case supposed to have nephritis, in which no autopsy was performed, death occurred in the third week, and the blood showed no lack of bactericidal power.

Non-fatal cases, five. In two markedly confluent cases, in which the blood was taken after full pustular development, there was a well-marked loss of power to inhibit growth, though not to the same degree as in the fatal cases. In one markedly confluent case, in which the eruption aborted at the outset of the pustular stage, the blood being taken at this time, the organisms were promptly killed. In one light case of discrete variola, in which the blood was taken in convalescence, and in one case of varioloid, in which it was taken at the height of the eruption, there was no loss of bactericidal power.

That the growth of the organisms in the serum was due to loss of bactericidal complement is shown by a series of experiments in which normal unheated serum was added in small amounts to the serum with weakened bactericidal action, and at once and effectually restored the full strength of this action. The exact quantities needed for such restoration were not estimated, but one-half volume was found sufficient in all cases.

The colon bacillus seemed in all cases in which it was tried to be more resistant than typhoid. Where both organisms were used and both showed increase, the colon increased faster than the typhoid, the plates being uncountable in some cases as much as eight hours before the typhoid plates became too crowded for numeration. In one case in which the typhoid bacillus was killed, though slowly, colon showed a decrease followed by a marked increase.

It was very striking to note in a number of cases that the count of colonies diminished for eight hours, and then increased as though the resistance had been exhausted in its efforts to kill off the organisms. It seemed to us that the comparatively small number of organisms dealt with gave rather more accurate ideas as to such minor changes than the introduction of so great a number as to overwhelm at once such resistance as was left.

In general, the blood of fatal cases taken on the fifth or sixth day, before the completion of the pustular development, showed no decrease of the bactericidal action against typhoid and colon, while those cases which passed this stage before death usually showed a loss of complement. Non-fatal cases in which the blood was taken late, after the completion of the pustular stage, but before complete convalescence, showed loss of complement, while those in which the blood was taken earlier, or after convalescence was complete, showed no such loss. The light cases and the varioloid showed no loss, as might be expected. In those cases which survived the variolic infection, and succumbed later to the effects of the secondary abscesses, the results were similar to those in Longcope's series, the disease being typically a wasting one, and the loss of complement being proportional.

In only one case of those examined in the early stages of the disease, a baby dying of the purpuric form, did we find a loss of complement. This child when admitted was markedly emaciated and apparently in a chronic condition of ill health, and it seems not improbable from the uniformity of the other results that had we been able to examine the blood

before the onset of variola we would have obtained similar results.

While we appreciate that the finding of a loss of complement towards colon and typhoid bacilli does not offer absolute proof that the body resistance towards the streptococcus group is changed, still, in the absence of present means for testing directly, we are compelled to base our conclusions on the results above stated.

Conclusions. — I. In cases dying from variola in the first five or six days of the eruption, before the completion of the pustular stage, there is practically no loss of complement.

II. In cases dying after this time, but still of the variolic infection, in the full pustular stage, there is a loss of complement, in general proportional to the extent of skin involved.

III. In cases dying after the disappearance of all symptoms directly attributable to variola, death being due to the long continued suppuration of secondary abscesses, the loss of complement is fairly uniform, and probably of similar origin with that described in wasting diseases.

IV. In light cases taken early or late, there is apparently no loss.

V. The fact set forth in another paper by us,¹ that the addition of normal serum with a high complement content to our anti-streptococcus serum inoculations, did not seem in any way to decrease the secondary infections from which streptococci could be cultivated, together with the general results as set forth in the present paper, lead us to the conclusion that the loss of complement has very little to do with the secondary streptococcus infections, but that, on the other hand, these infections, if sufficiently long continued, may lead to a very definite loss.

¹ Streptococci in variola. This number, p. 180.

ON THE CHEMISTRY OF THE CHROMATIN SUBSTANCE OF
THE NERVE-CELL.¹

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The importance of the chromatin substance of a cell for its various functions and for the processes of growth and regeneration is being constantly realized and its part in heredity to-day is firmly established. It is natural that the chromatin substance of the nerve-cell should be the center of attention to the neurologist. It is true that a few years ago much more light was expected from the study of the morphological elements of the nerve-cell than has really been obtained, but for this disappointment the efforts to establish the nature of the morphological elements of the nerve-cell and the character of their changes in physiological and pathological conditions should not be abandoned.

With the view of gaining some knowledge of the nature of Nissl's granules, and of their relationship to the chromatin of the nucleus, I undertook the study of the nucleoproteids of the brain.

It was established, especially by work done in Kossel's laboratory by Lilienfeld and by Lilienfeld and Malfatti, that chromatins belong to the class of substances commonly designated as nucleo-compounds.

The nucleo-compounds, however, differ greatly in their composition. The point of similarity of all of them lies in the fact that they are all derivatives of a phosphorized organic acid named nucleic acid. The chromatin may be a compound of a nucleic acid with a very simple proteid substance, a protamin like that of the heads of the spermatozoa of fishes, or it may be a compound with a more complex proteid substance, a histon. Further, a nucleic acid may combine with more than one different proteid substance to form a chromatin, as, for instance, that of the

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thymus; or it may combine with both proteids and carbohydrates to form a chromatin, as, for instance, that of the pancreas.

It is apparent that the distinction between different individual chromatins may be due to the nature of the different nucleic acids or to the other components entering in combination with the nucleic acid and thus forming the chromatin.

It was assumed, *a priori*, that in the higher organism the chromatins of different tissues are distinct in their functions. It was also assumed that distinction in function was to a certain degree caused by difference in chemical nature, and there was a certain tendency to ascribe the individual function of a chromatin to the nucleic acid present in its molecule.

That nucleic acids may possess a different chemical composition was assumed by Kossel, who was one of the first to undertake a thorough study of nucleo-compounds.

The components of nucleic acid, as far as known at present, are phosphoric acid, purin bases, pyrimidin bases, and a carbohydrate. A great variety of purin bases is known, but in the molecule of nucleic acids only two were found with certainty, namely, adenin and guanin. Of the pyrimidin group many bases were obtained synthetically, but only three were demonstrated in the molecule of nucleic acids, namely, thymine, cytosine, and uracil. Of all possible sugars the hexose and the pentose are those that most frequently occur in nature.

It is evident that nucleic acids may differ by the character of the substances of each of the three groups of substances which combine with phosphoric acid in order to form a nucleic acid.

Theoretically, it is true that there are many different combinations possible, but in nature only few distinct acids are supposed to exist. Thus at one time Kossel thought to have isolated an acid with only one purin base, adenin, and he designated that acid in distinction from the rest as Adenilic Acid. He soon discovered, however, that guanin also

could be found among the decomposition products of his acid.

Recently Bang has described an acid containing in its molecule only one of the purin bases, guanin, and named his acid Guanilic Acid.

Nucleic acids may also differ in the nature of their pyrimidin bases, and also by the presence or absence of these bases in their molecule. Indeed, Bang claimed that the nucleic acid of the pancreas contained no pyrimidin bases at all, and Osborne and Harris could detect in the nucleic acid of the wheat embryo only one of the three possible bases, namely, uracil.

Nucleic acids may also differ by the nature of the carbohydrate entering into their molecule.

Thus the question as it presented itself when I first undertook the study of the chemical nature of the chromatin of the brain was very complex. The first aim was to establish the nature of this chromatin in a general way. The analysis of the substance demonstrated that it belonged to the so-called "true nucleoproteids," to which group most of the chromatin known at that time belonged. It was, therefore, impossible to form any opinion as to the distinction of the nerve-chromatin from the chromatin of any other cell. I realized then that a thorough study of different components of the nucleoproteid was urgent. I also realized that the chemical composition of a cell constituent can serve to explain its part in the function of the cell only then, when it can be compared with the composition of analogous components of cells with a distinctly different function. However, at the time when I undertook the work on the nucleoproteids of the brain, the knowledge of the chemical nature of nucleoproteids in general was limited. I saw that in order to establish the part of the different components of the nucleoproteids of the brain they would have to be compared with analogous components of nucleoproteids of different origin.

As already stated, to the nucleic acid was ascribed more

importance than to any other component of chromatin, and my attention was, therefore, directed to that substance.

The attempts to obtain a nucleic acid of the brain by the methods then existing were futile. It was necessary to devise another process for obtaining the substance. The result of the efforts was a very simple process, by means of which nucleic acids could be obtained from nearly every tissue. This method made it possible to compare the elementary composition of different nucleic acids, and it was found that from that standpoint nucleic acids varied only a little.

A study of the different components was then undertaken. Attention was first directed to the purin bases. It was found that all the acids analyzed contained the same bases of that group, namely, adenin and guanin. Osborne and Harris made the same observation on the nucleic acid of the wheat embryo.

A study of the pyrimidin bases then followed. At the time the work was begun, only two bases of that group were known to be present in the molecule of nucleic acids — thymine, discovered by Kossel, existing only in acids of animal origin, and uracil existing only in acids of plant origin. Uracil was first discovered in the nuclein of yeast by Ascoli, who worked under Kossel, and later by Osborne in the acid of the wheat embryo. The following acids were analyzed in this direction: That of the thymus, spleen, fish spermatozoa, and yeast by Kossel, that of wheat embryo by Osborne and Harris, and Wheeler, and that of the yeast, spleen, pancreas, liver, testis, and brain by me. This work led to my obtaining a new base of the pyrimidin group. Simultaneously the same base was discovered by Kossel.¹ This work also led to the discovery of uracil in nucleic acids of animal origin. Uracil was found first by myself, and then by Kossel and Steudel.

The quantities of nucleic acid of the testis and of the brain available were too small to enable me to demonstrate uracil among its decomposition products.

¹ Kossel's publication appeared in Germany only a few days before my communication was made in Washington.

twenty-five per cent solution of sulphuric acid and digested in an autoclave for three hours between 150 and 175° C. The sulphuric and phosphoric acids were then removed by means of baryta water, and the filtrate concentrated at very low pressure. On standing over night a precipitate was formed which consisted chiefly of thymine and also of cytosine. To remove the latter, the thymine was recrystallized from a ten per cent solution of sulphuric acid. It was then dried and analyzed.

0.1200 gr. of the substance gave over 50 per cent KOH solution. 23.5 cc. of nitrogen at p — 760 mm — 24° C.

for $C_6 H_5 N_2 O_2$.

Calculated.	Found.
N — 22.22	— 22.52

The mother liquids of the thymine were then joined, the excess of barium removed by sulphuric acid, and the filtrate treated with a concentrated solution of picric acid. The solution was concentrated under low pressure to a small bulk and cytosine picrate was allowed to crystallize. The picrate was dissolved in hot water, filtered from the insoluble part and decomposed by means of sulphuric acid, toluol, and ether. When all the picric acid was removed, the solution was treated with a solution of barium hydrate until it ceased to react acid to congo, but reacted acid to litmus. The filtrate was concentrated under diminished pressure to a very small bulk and allowed to crystallize. Beautiful needles of the basic-cytosine sulphate formed immediately.

0.1450 gr. of the substance dried in Toluol bath gave 0.0588 gr. of $BaSO_4$.

for $4 (C_4 H_4 N_3 O) \cdot H_2 SO_4 \cdot 2 H_2 O$.

Calculated.	Found.
S — 5.53 per cent.	— 5.57 per cent.

Part of this salt was then transformed to the chlorplatinate.

0.1364 gr. of substance dried in Toluol bath gave 0.0421 gr. pt.

for 2 (C₄H₅N₃O) Pt Cl₄. 2 HCl.

Calculated.	Found.
Pt. — 30.84 per cent.	30.86 per cent.

The filtrate from the cytosin picrate was not sufficient to enable us to detect the presence of uracil in it.

Thus in the character of its pyrimidin bases the nucleic acid of the brain resembles those of other tissues, but only the nucleic acids of animal origin.

Whether or not all nucleic acids of animal origin are identical in their nature is, however, not yet known. It remains to establish the nature of the carbohydrate present in their molecule, and further to establish the proportions of the different components in the acids of different tissues. It seems probable from our present experience that the proportion of the purin bases to the pyrimidin bases, as well as the proportion of the different bases of each group, varies considerably in acids of different tissues. I hope to be in a position to establish this in the near future.

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ON THE AUTOLYSIS OF BRAIN TISSUE.¹

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Since it was established by the researches of Theobald Smith, by Salkowsky and his students, by the students of Hofmeister, and by Hedin that many organs contain proteolytic enzymes, attempts were made to apply these findings to the explanation of some pathological conditions. Thus, Fr. Müller demonstrated that lung tissue in pneumonia is restored to its normal condition owing to enzyme action; Martin Jacoby demonstrated that liver in phosphorus poisoning possesses a higher autolytic power than the normal organ. Very recently Ascoli demonstrated that the blood of patients with pneumonia undergoes in the course of the disease certain changes in its anti-enzymotic action. Methods were also devised for the study of organs subject to autolysis under aseptic conditions, and they were applied for the analysis of some morphological phenomena like the so-called fatty degeneration.

Autolytic enzymes were demonstrated in the following organs: muscle, liver, spleen, kidney, thymus, and lymphatic glands, and, of course, in the digestive glands. No experiments were made with the brain tissue, and yet it seemed of great importance to establish the presence or absence of proteolytic enzymes in that organ, for the following two reasons: first, there are known pathological conditions of the brain tissue analogous, to a certain extent, to the atrophy of the liver; and second, nerve tissue is known to undergo definite post-mortem morphological changes, which the study of the chemical transformation of the tissue might serve to explain.

The present publication represents the first of a series of experiments in that direction, and deals only with the question of the presence or absence of a proteolytic enzyme in the nerve tissue.

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Brains of large dogs were used for the experiment. They were carefully freed from the membranes, and thus were practically bloodless. Accurately weighed quantities of the finely divided brains were taken up in physiological salt solution, in 0.5 per cent sodium carbonate solution, and in 0.2 per cent acetic acid solution. Estimations of the nitrogen-distribution were made at the beginning of the experiment, and were repeated after six days of self-digestion.

The following estimations were made: Total nitrogen; nitrogen of the coagulable proteid; nitrogen of non-coagulable proteid; nitrogen of substances not precipitable by zinc sulphate (tabulated as Pepton and Amino compounds), and nitrogen as free ammonia.

With a view of ascertaining whether nerve tissue exercises an action inhibitory to the autolytic enzyme, brain tissue was allowed to act on heated brain tissue, and the nitrogen distribution in the mixture then determined.

EXPERIMENTAL PART.

Exp. I. — 1.53 grms. brain taken for Kjeldahl. 21.00 cc. N_{10} solution of H_2SO_4 required to neutralize = 0.0192 gm. N per gm. brain.

Exp. II.— 4.23 grms. brain + 25.5 cc. water + zinc sulphate to saturation.

Filtrate from zinc sulphate precipitate . . .	6.0 per cent.
Free ammonia	0.8 “ “

Exp. III.— 10.7 grms. fresh brain + 64 cc. saline. After seven days brought to boil, acetic acid added to coagulate, filtrate made up to 64 cc.

Filtrate from heat coagulum	18.3 per cent.
Filtrate from zinc sulphate precipitate . . .	8.7 “ “
Free ammonia	0.8 “ “

Exp. IV.— 5.92 grms. fresh brain, 35.5 cc. and 60 cc. of 2 per cent acetic acid.

After seven days neutralized, boiled, and acetic acid added to coagulate, made up to 35.5 cc.

Filtrate from heat coagulum	28.1 per cent.
Filtrate from zinc sulphate precipitate . .	13.0 " "
Free ammonia	0.8 " "

Exp. V.—5.1 grms. brain + 30 cc. water, boiled, cooled, and 5.5 grms. brain + 33 cc. water and acetic acid to 0.2 per cent added.

After seven days neutralized, brought to boil, acidulated with acetic acid, filtrate made up to 63 cc.

Filtrate from heat coagulum	22.1 per cent.
Filtrate from zinc sulphate precipitate . .	9.9 " "
Free ammonia	0.8 " "

Exp. VI.—8.9 grms. brain fresh + 53 cc. water and Na_2CO_3 to 0.5 per cent.

After seven days heated, acetic acid, etc.

Filtrate from heat coagulum	11.7 per cent.
Filtrate from zinc sulphate precipitate . .	6.5 " "
Free ammonia	0.8 " "

Exp. VII.—5.6 grms. brain + 33 cc. water, boiled, cooled, 5. grms. brain + 30 cc. water. Na_2CO_3 added to 0.5 per cent. After seven days heated, acetic acid, etc.

Filtrate after heat coagulum	11.7 per cent.
Filtrate after zinc sulphate precipitate . .	5.8 " "
Free ammonia	0.8 " "

TABLE OF RESULTS.

N in per cent of total Nitrogen.	At the beginning of experiment.	After digestion with physiological salt solution.		After digestion with 0.2% acetic acid.		After digestion with 0.5% sodium carbonate.	
		Without addition of heated brain.	With addition of heated brain.	Without addition of heated brain.	With addition of heated brain.	Without addition of heated brain.	With addition of heated brain.
Coagulable N.....	94.0	81.7	—	71.9	77.9	88.3	88.3
Albumose N.....	—	9.6	—	15.1	12.2	5.9	5.2
Pepton and Amino N.	6.0	8.7	—	13.0	9.9	5.8	6.5
Free ammonia N.....	0.8	0.8	—	0.8	0.8	0.8	0.8

It is seen from this table that nerve tissue possesses an autolytic power, that the autolysis is favored by the presence of acetic acid and is inhibited by the presence of alkali, that in the presence of acid the brain tissue exercises no inhibitory action on the process of brain-autolysis.

Thus in its proteolytic action nerve tissue does not differ from muscle tissue, or from the spleen and liver. The experiments also indicate that resistance of this tissue toward self-digestion depends upon the reaction of the tissue.

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ON THE DIGESTION AND SELF DIGESTION OF TISSUES
AND TISSUE EXTRACTS.¹

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It is well known that higher organisms cannot assimilate foreign proteid material. In order to be utilized by the organism it has to be decomposed into very simple crystalline compounds, and then reconstructed into tissue-substance. That the decomposition takes place in the gastro-intestinal tract through the action of enzymes is also a well-established fact.

Only within comparatively recent years was it demonstrated that enzymotic-proteolytic action takes place in monocellular organisms and within the cells of many tissues of higher plants and animals. This discovery led to numerous researches, in the course of which the following observations were made:

- (1.) That all living organs offer a resistance to the action of proteolytic enzymes of the digestive tract.
- (2.) That all living organs contain enzymes capable of digesting the proteids of that organ.
- (3.) That pepsin and trypsin are capable of decomposing all proteid material.
- (4.) That proteolytic enzymes of other tissues are, perhaps, specific in their action.
- (5.) That the resistance of tissues toward trypsin and toward pepsin is due, perhaps, to the presence in the blood of anti-enzymes.

These, of course, are only a few of the findings resulting from the many investigations on proteolytic enzymes. They are mentioned here for the reason that they naturally

¹ Read before the Third Annual Meeting of the American Association of Pathologists and Bacteriologists, under the title "On Intracellular Enzymes and Anti-Enzymes," Washington, May, 1903. Received for publication June 30, 1903.

lead to some new questions to which we attempted to find an answer.

An investigation of the following points was attempted :

(1.) Whether the inhibitory action of a tissue on trypsin or pepsin is exercised by its cells or by their soluble constituents.

(2.) Whether the tissue extracts exercise an inhibitory action on the proteolytic enzymes of other tissues — on the intracellular enzymes.

(3.) Whether the inhibitory action of the blood and tissues can be increased by means of immunization.

I. In regard to the first question, the following observations have been made. Hahn was the first to make the statement that not only blood, but also its serum, possessed anti-tryptic power. Landsteiner could find anti-tryptic power in the muscle plasma, and he also makes the statement, that the white of the egg shows inhibitory action on trypsin, while extracts from the liver, kidneys, and spleen are without any influence on the enzyme. Finally, Weiland explained the fact that the pancreas and stomach remain intact during life by the presence of an anti-enzyme.

The method employed by the first two writers was the usual gelatin test, namely, a comparison of the liquefying effect of the trypsin solution in gelatine, and of such a solution after the addition of different proportions of serum or tissue extract.

The principal object of our work was to establish the digestibility of tissue extracts, and the method of analysis was the following: Extracts of different organs and the white of the egg were treated with an extract of fresh pancreas. In the solution the total nitrogen, the nitrogen of the coagulable proteids, that of the non-coagulable proteid which could be precipitated by zinc sulphate, and the nitrogen of free ammonia were determined. From these figures the peptone nitrogen and the other nitrogenous organic substances were calculated by difference. These determinations were made at the beginning of the experiment, and then at intervals.

In the controls the tissue extracts were heated about one quarter of an hour in a boiling water bath, and only when cool was the solution containing the enzyme added.

The extract of the pancreas used in the experiments was prepared in the following manner: Fresh glands were chopped fine and allowed to stand with a 5 per cent solution of sodium carbonate and toluol over night at room temperature, after which the extract was filtered, and the filtrate kept in the refrigerator.

The digestibility of extracts of liver and spleen, blood, egg-white, also of the pancreatic extract itself unboiled, and one-half boiled, the other unboiled, was estimated. The experiments were very numerous and only part of them are tabulated here. (See Table I.)

The results of this series of experiments are all uniform, and all clearly show that unboiled extract is digested by pancreatic extract slower than one that had been heated previous to the experiment.

Further, the experiments show that the strength of the digestion depends upon the quantity of enzyme added.

The action of certain small quantities of pancreatic extract may be almost totally inhibited by unheated white of the egg; while the same proportion of the pancreatic extract added to previously heated egg-white causes marked digestion.

Finally, it is evident that in the course of digestion (Exp. VIII.) the enzyme action and the inhibitory action of the tissue extract are being exhausted.

TABLE I.

No. of Experiment.	Substance Used.		Time of Determination.	N in per cent of Total Nitrogen.			
	Heated.	Unheated.		Coagulable N.	Albumose N.	Peptone + Amino N.	Free NH ₃ N.
I.	160 cc. egg-white.	320 cc. 0.5 per cent Na ₂ CO ₃ , 1.25 grms. trypsin.	16 hrs. after 24 hrs. after 48 hrs. after	30.7 13.6 13.0	— — —	— — —	— — —
II.	160 cc. egg-white. 320 cc. 0.5 per cent Na ₂ CO ₃ , 1.25 grms. trypsin.	16 hrs. after 24 hrs. after 48 hrs. after	67.2 66.4 61.0	— — —	— — —	— — —
III.	50 cc. egg-white.	150 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 18 hrs. after 24 hrs. after	45.0 26.7 15.6	— — —	— — —	— — —
IV.	50 cc. egg-white. 150 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 18 hrs. after 24 hrs. after	45.0 40.0 33.7	— — —	— — —	— — —
V.	125 cc. egg-white. 375 cc. water.	25 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 48 hrs. after 72 hrs. after	74.4 18.9 14.0	— — —	— — —	— — —
VI.	125 cc. egg-white. 375 cc. water. 25 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 48 hrs. after 72 hrs. after	74.4 73.3 71.3	— — —	— — —	— — —
VII.	Tryptic digestion of egg-white after standstill had been reached.	20 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 48 hrs. after	14.9 7.0	42.9 42.9	42.3 50.0	9.0 10.0
VIII.	Tryptic digestion of egg-white after standstill had been reached. 20 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 48 hrs. after	15.0 7.2	43.1 43.3	41.9 49.5	9.0 10.5
IX.	600 cc. 0.5 per cent Na ₂ CO ₃ extract of liver.	200 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 48 hrs. after	60.5 1.7	28.5 42.3	17.0 56.0	0.5 3.2
X.	600 cc. 0.5 per cent Na ₂ CO ₃ extract of liver. 200 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 48 hrs. after	61.9 11.2	21.2 29.7	16.9 49.1	0.4 2.0
XI.	400 cc. 0.5 per cent Na ₂ CO ₃ extract of spleen.	150 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 24 hrs. after 72 hrs. after	54.3 25.4 9.3	17.2 25.3 27.1	28.5 49.3 53.6	0.6 1.2 3.0

TABLE I. — *Continued.*

No. of Experiment.	Substance Used.		Time of Determination.	N in per cent of Total Nitrogen.			
	Heated.	Unheated.		Coagulable N.	Albumose N.	Peptone + Amino N.	Free NH ₃ N.
XII.	400 cc. 0.5 per cent Na ₂ Co ₂ extract of spleen. 150 cc. 0.5 per cent Na ₂ Co ₂ extract of pancreas.	Beginning. 24 hrs. after 72 hrs. after	53.7 49.3 27.8	14.3 18.1 30.8	27.0 32.6 41.4	0.7 1.2 2.1
XIII.	36 cc. defibrinated blood of normal rabbit. 22 cc. saline.	40 cc. 0.5 per cent Na ₂ Co ₂ extract of pancreas.	Beginning. 24 hrs. after	88.1 31.7	6.4 37.3	5.5 31.0	0.4 1.2
XIV.	36 cc. defibrinated blood of normal rabbit, 72 cc. saline. 40 cc. 0.5 per cent Na ₂ Co ₂ extract of pancreas.	Beginning. 24 hrs. after	87.9 45.9	6.7 23.9	5.4 25.2	0.4 0.8

II. No experiments in regard to the second question are recorded to our knowledge. A suggestion that tissues may exercise some inhibitory action on the proteolytic enzyme of the liver is given by the experiments of Martin Jacoby, who demonstrated that the coagulable proteids of the lung-tissue cannot be digested by the liver extract.

The action of splenic extract on liver, on pancreatic extract, on blood, on white of the egg, was tried. The action of an extract of the liver on blood was also tried. A comparison of the self-digestion of the spleen when one-half had been heated previous to the experiment was made. A similar experiment was performed with the extract of the liver.

The results are tabulated below.

TABLE II.

No. of Experiment.	Substance Used.		Time of Determination.	N in per cent Total Nitrogen.			
	Heated.	Unheated.		Coagulable N.	Albumose N.	Peptone + Amino N.	Free NH ₃ N.
I.	100 cc. egg-white	25 cc. 0.2 per cent acetic extract of spleen. Acetic acid to 0.2 per cent.	Beginning.	88.8	7.7	3.5	0.4
			2 days after.	88.8	7.4	3.8	0.4
			10 d'ys after	85.4	5.8	8.8	
II.	100 cc. egg-white. 25 cc. 0.2 per cent acetic extract of spleen. Acetic acid to 0.2 per cent.	Beginning.	88.8	8.0	3.2	0.4
			2 days after.	89.0	7.5	3.5	0.4
			10 d'ys after	87.4	6.6	6.0	
III.	400 cc. 0.2 per cent acetic extract of pancreas.	200 cc. 0.2 per cent acetic extract of spleen.	Beginning.	68.4	13.9	17.7	0.7
			48 hrs. after	50.0	20.6	29.4	0.9
IV.	400 cc. 0.2 per cent acetic extract of pancreas. 200 cc. 0.2 per cent. acetic extract of spleen.	Beginning.	70.2	12.6	17.2	0.7
			48 hrs. after	56.7	16.7	26.6	0.8
V.	200 cc. 0.2 per cent acetic extract of spleen.	200 cc. 0.2 per cent acetic extract of spleen.	Beginning.	52.0	7.0	41.0	0.7
			12 hrs. after	48.0	8.0	44.0	
			60 hrs. after	37.0	10.0	53.0	1.4
VI.	400 cc. 0.2 per cent acetic extract of spleen.	Beginning.	47.4	9.9	40.7	0.5
			12 hrs. after	44.9	11.6	43.5	0.7
			60 hrs. after	38.0	12.0	70.0	1.5
VII.	200 cc. saline extract of liver.	200 cc. 0.1 per cent acetic extract of spleen. Acetic acid added to 0.1 per cent.	Beginning.	64.2	16.6	19.2	0.7
			24 hrs. after	50.0	17.9	32.1	0.8
VIII.	200 cc. saline extract of liver. 200 cc. 0.1 per cent. acetic extract of spleen. Acetic acid added to 0.1 cent.	Beginning.	64.3	15.7	20.0	0.5
			24 hrs. after	34.3	25.7	40.0	0.8
IX.	200 cc. saline extract of liver.	200 cc. saline extract of liver. Acetic acid added to 0.1 per cent.	Beginning.	82.7	4.0	13.3	1.6
			24 hrs. after	65.0	14.5	20.5	2.4
			48 hrs. after	55.5	18.4	26.1	3.2
X.	200 cc. saline extract of liver. Acetic acid added to 0.1 per cent.	Beginning.	82.7	4.0	13.3	1.6
			24 hrs. after	55.5	20.0	25.5	3.2
			48 hrs. after	46.0	15.0	38.3	5.0

It is seen from these experiments that white of egg and pancreatic extract are easier digested by splenic extract after previous boiling — and further that when one-half of the splenic extract has been heated, the quantity of digested proteid is smaller than in the case when all the extract was not heated. The same is observed with regard to extracts of liver, and the same result occurs when the spleen is allowed to act on the liver, or on the blood, or when the liver is allowed to act on the blood. It thus may seem that the enzymes of the spleen and of the liver possess the power to digest unchanged proteid and do not act on heated proteid. But on more careful analysis of the experiments another explanation to this observation may be offered, namely, the proportion of enzyme to the total proteid material is smaller where one-half of the extract used for experiment has been boiled. It is even probable that the acid extract of the spleen or liver exposed to self-digestion exercises its maximum digestive power. It may also be probable that the inhibitory action of the extract is destroyed by the acid, and if this assumption is correct, then it follows that the acid extract contains only the enzyme, and that by heating one-half of the extract used for experiment, part of the enzyme would be destroyed, and thus only a retardation of the digestion could be expected.

The following experiment demonstrates that a tissue left in contact with 0.2 per cent of acetic acid loses its anti-proteolytic action. It was performed on rabbits' blood:

TABLE III.

No. of Experiment.	Substance Used.		Time of Determination.	N in per cent of Total Nitrogen.			
	Boiled.	Unboiled.		Coagulable N.	Albumose N.	Peptone + Amino N.	Free NH ₃ , N.
I.	12.5 cc. defibrinated blood of pancreatized rabbit. 87.5 cc. saline. Acetic acid to 0.2 per cent after 24 hours at room temperature.	Na ₂ CO ₃ to 0.5 per cent. 10 cc. 0.5 per cent. Na ₂ CO ₃ extract of pancreas.	Beginning. 48 hours after.	87.5 43.3	4.2 30.9	8.3 25.8	0.8 2.0
II.	12.5 cc. defibrinated blood of pancreatized rabbit. 87.5 cc. saline. Acetic acid. 0.2 per cent after standing 24 hours at room temperature. Na ₂ CO ₃ to 0.5 per cent. 10 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 48 hours after.	88.7 50.0	2.6 25.7	8.7 24.3	0.8 1.7

Thus in the pancreatic extract and in the white of the egg an inhibitory action against the intracellular enzymes is demonstrated. In the liver, spleen, and blood this inhibitory action could not have been demonstrated; perhaps, for the reason that acetic acid destroyed this property of the neutral extracts.

III. In regard to the third question, Achalme reported the results of experiments on guinea-pigs. These animals, after treatment with trypsin, were rendered immune against the enzyme, and their blood possessed a higher anti-tryptic action than that of normal animals. Later, Landsteiner attempted to immunize rabbits with trypsin, but his attempts to increase the normal anti-tryptic action of their blood were without any success. In our experiments rabbits received on alternate days for eight weeks subcutaneous injections of pancreatic extract, in doses beginning with 0.5 cc. and gradually increasing to 2.0 cc. of the extract each injection. The

defibrinated blood (heated and unheated) of these animals was then tested in regard to its resistance toward the action of trypsin. For control the blood of normal animals was used. The results are given in Table IV.

TABLE IV.

No. of Experiment.	Substance Used.		Time of Determination.	N in per cent Total Nitrogen.			
	Heated.	Unheated.		Coagulable N.	Albumose N.	Peptone + Amino N.	Free NH ₃ N.
I.	18 cc. defibrinated blood of pancreatinized rabbit. 36 cc. saline.	20 cc. 0.5 per cent Na ₂ CO ₃ Pancreatic extract.	Beginning. 24 hrs. after	90.1 25.8	5.7 35.7	4.2 38.5	0.5 4.0
II.	18 cc. defibrinated blood of pancreatinized rabbit. 36 cc. saline. 20 cc. 0.5 per cent Na ₂ CO ₃ pancreatic extract.	Beginning. 24 hrs. after	90.1 76.7	5.7 11.4	4.2 11.9	0.5 2.0
III.	12.5 cc. defibrinated blood of pancreatinized rabbit. 87.5 cc. saline.	10 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 48 hrs. after	79.0 44.0	11.0 14.0	10.0 40.0	0.9 2.5
IV.	12.5 cc. defibrinated blood of pancreatinized rabbit. 87.5 cc. saline. 10 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 48 hrs. after	79.0 74.0	11.0 13.0	10.0 13.0	0.9 1.2
V.	36 cc. defibrinated blood of normal rabbit. 72 cc. saline.	40 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 24 hrs. after	88.1 31.7	6.4 37.3	5.5 31.0	0.4 1.2
VI.	36 cc. defibrinated blood of normal rabbit. 72 cc. saline. 40 cc. 0.5 per cent Na ₂ CO ₃ extract of pancreas.	Beginning. 24 hrs. after	87.9 45.9	6.7 28.9	5.4 25.2	0.4 0.8
VII.	10 cc. defibrinated blood of normal rabbit. 50 cc. saline.	20 cc. 0.2 per cent acetic extract of spleen. Acetic acid to 0.1 per cent.	Beginning. 4 days after	90.0 75.9	5.9 14.1	4.0 10.0	0.7 1.3

TABLE IV. — *Continued.*

No. of Experiment.	Substance Used.		Time of Determination.	N in per cent of Total Nitrogen.			
	Heated.	Unheated.		Coagulable N.	Albumose N.	Peptone + Amino N.	Free NH ₄ N.
VIII.	10 cc. defibrinated blood of normal rabbit. 50 cc. saline. 20 cc. 0.2 per cent acetic extract of spleen. Acetic acid to 0.1 per cent.	Beginning. 4 days after	89.8 50.7	6.4 32.3	3.8 17.0	0.7 1.1
IX.	10 cc. defibrinated blood of pancreatinized rabbit. 50 cc. saline.	20 cc. 0.2 per cent acetic extract of spleen. Acetic acid added to 0.1 per cent.	Beginning. 4 days after	91.8 81.2	4.7 9.1	3.5 9.7	0.6 0.9
X.	10 cc. defibrinated blood of pancreatinized rabbit. 50 cc. saline. 20 cc. 0.2 per cent acetic extract of spleen. Acetic acid added to 0.1 per cent.	Beginning. 4 days after	91.8 63.7	4.7 22.1	3.5 14.1	0.6 1.1
XI.	10 cc. defibrinated blood of normal rabbit. 50 cc. saline.	20 cc. 0.2 per cent acetic extract of liver. Acetic acid to 0.1 per cent.	Beginning. 4 days after	90.0 84.3	6.1 8.6	3.9 7.1	0.6 0.9
XII.	10 cc. defibrinated blood of normal rabbit. 50 cc. saline. 20 cc. 0.2 per cent acetic extract of liver. Acetic acid to 0.1 per cent.	Beginning. 4 days after	90.0 67.8	6.1 22.1	3.9 10.1	0.6 1.2
XIII.	10 cc. defibrinated pancreatinized blood of rabbit. 50 cc. saline.	20 cc. 0.2 per cent acetic extract of liver.	Beginning. 4 days after	92.0 87.5	4.4 5.3	3.6 7.2	0.6 0.9
XIV.	10 cc. defibrinated blood of pancreatinized rabbit. 50 cc. saline. 20 cc. 0.2 per cent acetic extract of liver.	Beginning. 4 days after	92.0 76.3	4.4 4.3	3.6 18.4	0.6 1.2

This table shows that the blood of the immunized rabbits offers a markedly higher resistance toward tryptic digestion than that of the normal animal.

The behavior of the same blood toward splenic and

hepatic extracts was not much different from that of normal blood. This also may have been due to the fact that the inhibitory power against the enzyme was destroyed by the presence of the acetic acid.

The work on immunization of animals against enzymes of the liver and of the spleen is now in progress. We shall also attempt to ascertain whether or not the autolytic power of the liver and other organs can be checked by means of immunization.

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THE AGGLUTINATION OF THE PNEUMOCOCCUS WITH CERTAIN NORMAL AND IMMUNE SERA.¹

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In the course of some experiments on pneumococcus infection in the immunized rabbit, it was important to have some criterion, such as the agglutination reaction, of the degree of immunization developed in the animals. The methods used by other observers to determine the pneumococcus agglutination were tried. Some of these were wholly unreliable; others were suggestive, but uncertain; none were found trustworthy or sufficiently delicate when practised as a routine. After a long series of experiments, the chief faults in these previous methods were determined and in a large measure eliminated. As a result of these studies, I devised a new technic which has given more precise reactions than any of the methods hitherto used and which has proved sufficiently delicate to be, I believe, of broader and more practical service.

Previous methods. — The susceptibility of some animals, such as the rabbit, to pneumococcus infection as compared with the relative insusceptibility of certain other animals, such as the dog, has been determined from the results of animal inoculation. These observations were accompanied and followed by more accurate studies of the action of the sera of different animals on the growth of the pneumococcus. Metchnikoff, Behring and Nissen, Kruse and Pansini, Washbourne and Eyre, and others have noted that in general the blood serum of the rabbit has practically no bactericidal action on the pneumococcus; that of the guinea-pig varies,

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some exerting slight action; while the blood sera of cats, dogs, rats, squirrels, sheep, and horses are bactericidal in varying degrees, and less favorable to the growth of pneumococci. Kruse and Pansini noted especially the more marked formation of chains and bunches in the sera of the less susceptible animals. These variations in the growth of the pneumococcus have been further studied by Bezancon and Griffon, and also by Huber. The growth in bunches and chains was considered so marked and characteristic in the sera of specifically immunized animals that these observers describe it as a pneumococcus agglutination. Bezancon and Griffon inoculated one to two cubic centimeters of clear undiluted blood serum with pneumococci and observed the character of the growth at the end of fifteen or sixteen hours' incubation. The growth formed a membrane at the bottom of the tube, composed of bunches and chains, and this so-called agglutination was easily seen with the unaided eye; or the growth clouded the medium, and the small bunches and short chains could only be seen with the microscope. This method was applied clinically by them to one hundred and eighty-six patients — sixty-four of undoubted pneumococcus infection, twenty-two uncertain cases, and one hundred controls. In the serum from cases of pneumococcus infection the growth in bunches and chains, such as was found in the immune sera, could be distinguished easily and constantly from the fine clouding produced by the growth in the controls of normal serum. They, therefore, considered this so-called agglutination of practical diagnostic value. Although these observers noted this reaction in dilutions of immune sera up to one to fifty, they found that the results were very uncertain.

Neufeld studied the agglutinative action of the serum of immunized animals on pneumococcus cultures in broth by the usual methods, and was thus the first to note typical agglutination reactions in various dilutions, both microscopically and macroscopically. The maximum observed was one to fifty. He found that the agglutination took place in one-quarter to one-half hour after the mixtures were made. In order to

obtain good reactions the serum must be drawn from highly immunized animals and must be fresh. If allowed to stand for two weeks or even less he found that it was no longer active. He also found the activity of the sera from different animals to vary greatly and the reaction was often uncertain. In Neufeld's experiments different cultures of pneumococci agglutinated equally well. Compared with the typhoid agglutination, Neufeld found that the pneumococcus reaction differed in some respects. Thus he claimed that heated (100° C. for 3 hours) pneumococcus cultures agglutinated as well as the fresh. Furthermore, normal sera from the rabbit or man, in his observations, always failed to agglutinate pneumococcus cultures. These differences, as will subsequently be shown, are only apparent, not real, and are due to the lack of delicacy in the method.

Criticism and corroboration of the work of other observers. — However accurate may be the observations previous to Neufeld on the above noted growth of the pneumococcus in chains and bunches, and however much these may suggest the existence of special immune substances in certain sera, they were, nevertheless, based on rather crude experiments, allowing of much latitude in their interpretation. In all such experiments with bacteria the special selection attained by the micro-organism from its previous environment and its ability to adjust itself to sudden changes, are variable and disturbing factors not easily eliminated. But, aside from this, there are other and often indeterminate conditions, such as the amount of oxygen absorbed by the serum, its sugar content, reaction, and so on — conditions to which the pneumococcus is extremely sensitive, and which in the ordinary artificial media suffice to bring about many of the above noted phenomena in the growth of the pneumococcus.

I have grown the pneumococcus in the fresh, undiluted sera of the cat, dog, sheep, bullock, horse, and man, and in various ascitic and pleuritic fluids, and have been unable to note any consistent variation in the character of the growth which I could safely correlate with any special resistance or lack of susceptibility to infection in the animal. Furthermore, I have

grown the pneumococcus in sera from immunized rabbits and obtained the fine clouding noted in other sera. This, however, may be considered more or less exceptional, for it is certain that short chains or small clumps were more often found in the sera of highly immunized animals. On the other hand, a rapid growth in normal rabbits' (or other) serum under most favorable conditions — as to oxygen, sugar, and so forth — frequently produced a flocculent precipitate which gave a voluminous sediment, similar to the appearances Bezancon and Griffon describe as occurring only in immune serum. These facts suffice to indicate the extreme care necessary in making observations in this field and the great chance of error.

Neufeld's observations of the typical agglutination of pneumococcus cultures by immune sera was easily confirmed in my experiments. For example, the serum from two immunized rabbits¹ when mixed with forty-eight to seventy-two-hour broth cultures of pneumococci in dilutions of one to ten and one to twenty, showed a clumping not present in the one to sixty or control tubes; but in dilutions of one to one the rapid lysis obscured the agglutination phenomena.

The lysis occurring in dilutions of one to one or less was studied in the hanging drop and by stained microscopic preparations. With methylene blue the cells stained very faintly, and with the gentian violet copper sulphate stain recommended by Hiss, empty capsules, degenerated fragments of cells in capsules, in clumps or singly were found. In short, these were the changes first described by Radziewsky in his studies of the Pfeiffer phenomenon with the pneumococcus, and later by Neufeld. The swelling of the cells which one sees in the hanging drop and noted by the above cited observers as a first stage in the degeneration, it seems probable, is the usual normal development of the capsule in the presence of the serum described by Hiss, who brought out the fact that capsules may be demonstrated on pneumococci growing in artificial media, if only a drop of serum be

¹ These animals, 116 and 117, were immunized in the course of two weeks by inoculation with boiled cultures of pneumococci, 5 cc. subcutaneous and 2½ cc. intravenous, and with living cultures, 1 cc. subcutaneous and 1 cc. intravenous. At the site of inoculation one animal developed a subcutaneous abscess from which it recovered.

added in preparing the coverglass. This lysis of the pneumococcus cells obscuring the agglutination was, I found, easily eliminated by heating the serum to 56° C. for one hour, and thus destroying the complement. With old sera this was unnecessary, as the complement had already degenerated.

I have thus been able to corroborate the general observations of others as to the bacteriolytic and agglutinative action of specific immune sera upon the pneumococcus; but as a practical routine procedure for determining with delicacy the activity of different normal or immune sera, the methods were complete failures in my experience. This was due to many variable and often indeterminate conditions, but chiefly to the difficulty of distinguishing slight reactions and to the very considerable immunity which had to be developed by the animals before the presence of agglutinins in the blood serum could be detected. The great drawbacks to precise observation were the biological condition of the pneumococci and the small quantity of growth present in the usual broth cultures. It was hoped that these difficulties would in a measure be overcome, and that better and more precise results might be obtained, by concentrating the cultures at an early stage of the growth so that a greater number of fresh cells would be present in the same amount of fluid.

The new technic. — A peptone broth made from meat infusion which had been carefully neutralized before boiling has, in my experience, proved an optimum artificial fluid medium for growing the pneumococcus, especially if allowed to stand in the cold and absorb oxygen. In a flask containing approximately two hundred cubic centimeters the maximum growth is usually reached in from twenty-four to thirty-six hours.¹ At this stage the culture is centrifugalized,

¹ If glucose be added to the media, this maximum growth is reached much earlier, and there is an increasing quantity of precipitate formed as the growth continues. The precipitate increases the amount of sediment, and carries down many of the pneumococcus cells. Such cultures should be centrifugalized before much sediment appears, and only the top whitish layer used, as this is composed chiefly of pneumococcus cells. My experience with the glucose growth as compared with the growth in plain broth seems to indicate that if taken at the best stage the glucose growth gives the more delicate reactions; but the growth in plain broth, without glucose, gives

the clear fluid decanted, and the sediment shaken with about fifteen cubic centimeters of isotonic (.85 per cent) salt solution. The few clumps not broken up sink rapidly, leaving a dense, finely divided suspension of pneumococcus cells less than forty-eight hours old.¹ Dilutions with the sera to be studied may then be made in small, slender tubes, and observed for some twelve to eighteen hours at 37° C. The more marked reactions may be complete in five to six hours or less, but twelve or more hours are often required to bring out the more delicate tests.

The serum was at first heated to prevent lysis, but as the reactions were studied in so much higher dilutions, this was abandoned and the fresh serum used, as is customary in typhoid and other agglutinations. Sera stored in the ice-box were found to be active for four or more months; the lytic power was gone, and the agglutination did not appear in high dilution, but in low dilution immediate and complete reactions took place. Previous methods were not sufficiently accurate to establish this, and Neufeld drew the erroneous conclusion that in two weeks the pneumococcus agglutinin had degenerated.

In order to eliminate the growth of the organisms, the salt solution suspension was heated for one hour at 56° C.² Comparative tests of this procedure were made with strongly active sera. In low dilutions the reactions were marked and there was little difference apparent; but in high dilution the heated cells did not show the clumping as well as the unheated cells. In the low dilution the agglutination appeared early, before much growth had developed, and in the high dilutions the amount of growth was slight, so that it rarely interfered with accurate observation. Occasionally, however, the pneumococcus cells, when very fresh and in large numbers, developed with great rapidity, clouding the mixtures, and forming a flocculent precipitate which gave in a

possibly the more stable and better average. This is doubtless due to the rapid degeneration of the cells in glucose cultures.

¹ If much older growths or suspensions be used, the cells settle too rapidly, rendering the comparison of tests with controls difficult and uncertain.

² An exposure of twenty minutes to 52° C. usually kills the pneumococcus.

few hours a voluminous sediment.¹ This might easily be taken for an agglutination, were it not that the growth is always marked and the control mixtures with normal serum have the same appearance. The fact that this may occur is important, as suggesting the chances of error and as indicating the necessity of control mixtures with normal sera, if observations are to be made after much growth has taken place.

Results obtained with the new technic. — I have studied the agglutination of the pneumococcus in the blood serum from various normal animals and from a large number of specifically immunized rabbits by means of this new technic. It is not necessary to give in detail all the experiments; only such have been selected as serve to demonstrate the comparative value of the method and certain other points of interest.

Rabbits 140 and 141 were immunized in the following manner:

12/19. 5 cc. dense suspension dead pneumococcus cells, subcutaneous.

12/22. 5 cc. moderately dense suspension dead pneumococcus cells, intravenous.

12/24. 5 cc. luxuriant broth culture heated to 100° C., subcutaneous.

12/26. 3 cc. luxuriant broth culture heated to 100° C., intravenous.

12/31. .5 cc. luxuriant broth culture, fresh, subcutaneous.

1/5. .2 " " " " " "

1/8. 1.5 " " " " " intravenous.

1/17. Rabbit 140 died from pneumococcus infection.

Rabbit 141 lived, and January 14 was bled from the ear vein and the serum used for agglutination tests.

¹This was most marked when the serum and suspension of pneumococci were very fresh, and when stored in the ice-box a short time, absorbing considerable oxygen. By warming the fluids, driving off the oxygen, and by using deep, slender tubes, the difficulty was, in a measure, overcome. As will subsequently be shown, a precipitation may develop in the mixtures of sera with salt-solution suspensions. This is, however, a more or less specific phenomenon, and not to be confused with the ordinary precipitation which often occurs in sera and in some artificial media from pneumococcus growth.

1/21. A serum broth culture of the same race of pneumococcus used in the process of immunization, but recently isolated from an animal, was mixed with serum of Rabbit 141 in the following experiment:

Serum.	Salt Sol.	Culture.	Dilution 37° C.	12 hours.
.3 cc.	0	.3 cc.	1-1	+++
.1 "	.2 cc.	.3 "	1-6	++
.05 "	.25 "	.3 "	1-20	+ slight
Control	.3 "	.3 "	0	0

2/2. A dense, fine suspension in salt solution (.85 per cent) of centrifugalized glucose broth culture sediment, less than forty-eight hours old, was substituted for the serum broth culture.

Serum.	Salt Sol.	Pnc. Suspen.	Dilution 37°.	2 hours.	5 hours.	12 hours.	20 hours.
1/5 cc.	qs. ad.	.5 cc.	1. cc.	1-7½	++++	Prac. Complete	Complete
1/10 "	" "	.5 "	1. "	1-15	++++	" "	"
1/15 "	" "	.5 "	1. "	1-22½	++++	" "	"
1/30 "	" "	.5 "	1. "	1-45	++	++	"
1/60 "	" "	.5 "	1. "	1-90	?	+	"
1/45 "	" "	.5 "	1. "	1-67½		Complete	
1/90 "	" "	.5 "	1. "	1-135		"	
1/180 "	" "	.5 "	1. "	1-270		"	
Control	.5 "	1. "	0		Slight sediment only.	0	

2/6. Experiment continued with fresh culture suspensions.

Serum.	Salt Sol.	Pnc. Suspen.	Dilution 37°.	1 hour.	4 hours.	20 hours.
1/180 cc.	qs. ad.	.5 cc.	1. cc.	1-270	0 0	Prac. Complete
1/300 "	" "	.5 "	1. "	1-450	0 0	++
1/450 "	" "	.5 "	1. "	1-675	0 0	+ ?
1/900 "	" "	.5 "	1. "	1-1,700	0 0	0
Control	.5 "	1. "	0	0	0	0

2/26. Experiment was repeated. Centrifugalized sediment from plain broth culture was shaken in .85 salt solution and divided into two portions, one of which, B, was heated 52° C., one-half hour.

Serum.	Salt Sol.	Cult. Suspen.	Dilution 37°.	5 hours.	18 hours.
<u>A</u>					
.05 cc.	qs. ad. .25 cc.	.5 cc.	1-15	?	+++
.01 "	" " .25 "	.5 "	1-75	?	++
.01 "	" " .25 "	.5 "	1-75	?	++
.002 "	" " .25 "	.5 "	1-375	0	+
.002 "	" " .25 "	.5 "	1-375	0	+
.0004 "	" " .25 "	.5 "	1-1,875	0	0
<u>B</u>					
.01 "	" " .25 "	.5 "	1-75	0	20
.01 "	" " .25 "	.5 "	1-75	0	20
.002 "	" " .25 "	.5 "	1-375	0	0
.002 "	" " .25 "	.5 "	1-375	0	0
.0004 "	" " .25 "	.5 "	1-1,875	0	0

Controls:

	Salt Sol.	Cult. Suspen.	Dilution, 37°.	5 hours.	18 hours.
<u>A</u>					
0	.25 cc.	.5 cc.	0	0	0
Ascites Fld.					
.05 cc. qs. ad. .25 "		.5 "	1-15	0	0
.05 " " " .25 "		.5 "	1-15	0	0

Careful study of these reactions under the microscope in hanging drop and stained preparations brought out many points of interest. The cells, morphologically and tinctorially normal, are usually single or in pairs, rarely in clumps; whereas the clumps are composed chiefly of poorly staining cells whose morphology is often made out with difficulty. It seems fair to assume that the agglutinin acts principally on the degenerating cells when present. The intact cells develop and, if sufficient nutriment is present, cloud the fluid as usual. On the other hand, normal fresh cells may be rapidly clumped in low dilutions of very active immune sera, and the reaction may be complete in a few minutes under these circumstances.

It is evident from the above cited and other similar experiments that the agglutinative action of immune sera with heated pneumococcus may not be detected in as high dilutions as with fresh, unheated cells, and that the pneumococcus agglutinin is fairly stable and does not disappear from the immune sera for a considerable time. In short, the pneumococcus agglutinin conforms to the general laws governing typhoid and other agglutinins, except as regards the action

on motility. The greater delicacy sometimes seen in the reactions on glucose growths is also suggested in the above cited and other series of experiments.

The agglutinative action of sera from normal animals on the pneumococcus. — The normal serum of the rabbit always failed to agglutinate the pneumococcus cells. The growth of the organisms with marked formation of precipitate gave a flocculent sediment in some instances, but this was not considered a positive reaction. The normal blood sera of the cat and dog also failed to agglutinate. Normal bullocks' serum, however, gave some very marked reactions, even in dilution of one to fifty. Ascites fluids tested failed to agglutinate in dilutions of one to five and one to fifteen; but normal human sera gave reactions in dilution up to one to ten in less than eighteen hours; one to thirty failed to agglutinate.

The agglutinative action of the blood serum from pneumonia patients on pneumococci. — Through the kindness of Dr. F. C. Wood, I obtained a specimen of blood from a case of lobar pneumonia. The serum gave a marked reaction in dilution of one to ten after five hours' incubation. The quantity of serum was small, and it was not possible to test the reaction further. Two specimens were obtained from two other cases of pneumonia through Dr. G. B. Rhoades. These yielded enough serum to retest my results.

	Dilution 37°.	4 hours.	6 hours.	24 hours.
I. Pneumonia Serum	1-9	?	++	+++
	1-18	?	+	++
	1-27	o	+ ?	++
Controls :				
Salt Sol.	o	o	o	o
Ascites Fluid	1-3	o	o	o
	Dilution 37°.	3 hours.	5 hours.	24 hours.
II. Pneumonia Serum	1-10	?	++	+++
	1-20	o	+	+++
	1-50	o	o	++
	1-100	o	o	? slight
Controls :				
Salt Sol.	o	o.	o	o
Normal blood sera, rabbit	1-8	o	o	o
" " " man	1-10	o	o	+
	1-50	o	o	o

The close relation that has always been thought to exist between the pneumococci and the streptococci suggested testing the action of pneumococcus serum on suspensions of streptococcus cells in salt solution and, in reverse fashion, the action of streptococcus serum on pneumococci.

The agglutinative action of pneumococcus serum on streptococci. — A strongly active serum from Rabbit 141, immunized against the pneumococcus, was added in various dilutions to a salt solution suspension of streptococcus growth. These mixtures settled rapidly, and the growth of the streptococcus in chains gave a granular or flocculent sediment. This appearance was more marked in the tests than in the controls with normal serum, but the difference was slight and not considered definitely significant.

The agglutinative action of streptococcus serum on pneumococci. — The serum from a rabbit immunized with centrifugalized cultures of streptococci was added to the salt solution suspensions of pneumococcus cells in the following dilutions:

Streptococcus Serum.	Salt Sol.	Salt Sol. Susp. Pneumococci.	Dilution 37°.	10 hours.
.25 cc.	0	.25 cc.	1-1	++ +
.1 "	qs. ad. .25 cc.	.25 "	-5	++
.05 "	" " .25 "	.25 "	1-10	+
.0125 "	" " .25 "	.25 "	1-40	0

There was, apparently, considerable growth and some precipitate formed even as early as ten hours, and as this was also found in the controls of normal rabbits' serum, the experiment was supplemented by tests on the pneumococcus cells heated to 52 degrees for one-half hour, thus preventing growth. In this experiment agglutination took place in dilutions up to one to ten after eighteen hours' incubation.

Pneumococcus precipitin reaction. — Neufeld is, I believe, the only observer who has described a pneumococcus precipitin reaction. He found that if normal rabbits' bile is added to a fresh pneumococcus culture in the proportion of

one drop to one cubic centimeter of culture, the media becomes clear and the cells are no longer demonstrable. If such cleared culture is added to the serum of highly immunized rabbits, a precipitation takes place. The greatest dilution at which the reaction developed was one to nine. This precipitate Neufeld found was composed of highly refracting granules of variable size and easily observed under the microscope. These observations of Neufeld I was able to corroborate with the serum from only one of my immunized animals; but by dissolving the pneumococcus cells in the dense salt solution suspensions with normal rabbits' bile, I obtained a clear sterile fluid which formed voluminous precipitates with several of my pneumococcus sera in various dilutions.

Pneumococcus		Solu. (bile)		Dilution 37°.	1 hour.	12 hours.
Serum.	Salt Sol.	Pneumococcus.	Pneumococcus.			
.25 cc.	0	.25 cc.	.25 cc.	1-1	+	+++++
.1 " qs. ad.	.25 cc.	.25 "	.25 "	1-5	+	+++++
.05 " " "	.25 "	.25 "	.25 "	1-10	0	+++
.02 " " "	.25 "	.25 "	.25 "	1-25	0	++
.01 " " "	.25 "	.25 "	.25 "	1-50	0	+
.0075 " " "	.25 "	.25 "	.25 "	1-66.6	0	+?
.005 " " "	.25 "	.25 "	.25 "	1-100	0	0
Controls	.25 "	.25 "	.25 "	0	0	0

Pneumococcus Serum.		Normal Bile and Broth.		Dilution 37°.	1 hour.	12 hours.
Serum.	Salt Sol.	Bile and Broth.	Bile and Broth.			
.25 cc.	0	.25 cc.	.25 cc.	1-1	0	0
Pneumococcus Serum.		Normal Bile and Salt Sol.		Dilution 37°.	1 hour.	12 hours.
Serum.	Salt Sol.	Bile and Salt Sol.	Bile and Salt Sol.			
.25 cc.	0	.25 cc.	.25 cc.	1-1	0	0

In order to simplify the procedure and remove all question of the part played by the bile, another method was adopted. The centrifugized fresh pneumococcus cells were, in order to increase the plasmoptysis, first shaken with 17 per cent salt solution; then this was made isotonic (.85 per cent) by the addition of water, and filtered. The filtrate¹ thus obtained

¹ It may be well to suggest that the precipitate formed from the media as a result of the growth may be partially redissolved by the different salt solutions, and some may be retained in the filtrate. How this, if true, might influence the significance of the results of these precipitin reactions is at present uncertain.

gave the precipitin reaction with immune sera in dilutions up to one to fifty, as did the bile solutions. The filtrates prepared in this way varied greatly, but even the weakest showed some precipitate when mixed with the very active immune sera.

Pneumococcus Immune Serum.	Salt Sol.	Ext. Pneumoc.	Salt Sol.	Dilution 37°	¼ hr.	1 hr.	5 hrs.	16 hrs.
.25 cc.	0		.25 cc.	1-1	++	+++	++++	—
.05 " qs. ad.	.25 cc.		.25 "	1-10	+	++	+++	—
.0125 " "	.25 "		.25 "	1-40	0	0	+	+
.005 " "	.25 "		.25 "	1-100	0	0	0	++?
.00125 " "	.25 "		.25 "	1-400	0	0	0	0

Serum from Human Pneumonia.

1 part	0		1 part	1-1	+		++	+++
.05 cc.	qs. ad.	.25 cc.	.25 cc.	1-10	0		+	++
.01 " "	" "	.25 "	.25 "	1-50	0		?	+

Normal Human Serum.

1 part	0		1 part	1-1	0		?	++
.05 cc.	qs. ad.	.25 cc.	.25 cc.	1-10	0		?	+

Normal Bullock Serum.

.25 cc.	0		.25 cc.	1-1	0		?	+
.05 " "	qs. ad.	.25 cc.	.25 "	1-10	0		0	0

Normal Rabbit Serum.

.25 cc.	0		.25 cc.	1-1	0		0	0
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In these and other similar experiments the precipitin reaction was obtained with pneumococcus immune rabbit serum in dilutions of one to forty or more; one to one hundred being doubtful. In tests of the serum from a case of lobar pneumonia precipitates were formed with dilutions of one to one in less than one-half hour incubation, with dilutions of one to fifty in sixteen hours, and with dilutions of one to one hundred very slight after twenty-four hours' incubation. Normal human serum, diluted one to ten, gave the reaction in sixteen hours; normal bullocks' serum, diluted one to five, in sixteen hours. With a weaker filtrate no reactions were

noted with normal human serum, but the normal sera of the bullock, cat, and dog each gave the reaction in dilutions of one to one. With very active immune serum, diluted one to ten, a precipitate was formed with this filtrate after three hours' incubation.

Normal rabbit serum always failed to give any reaction with these filtrates. Once, however, a temporary clouding was noted as a ring at the junction of the two fluids in a one to one dilution. This disappeared on shaking the tube.

Serum from a rabbit immunized against streptococcus gave a marked reaction with the strongest filtrate in dilutions of one to one after ten hours' incubation. This was confirmed by subsequent tests with another filtrate, but failed to develop with the very weak filtrates.

Briefly summarized the results of my experiments show :

1. That peculiar growth phenomena occurring in pneumococcus cultures in serum are not reliable tests of the presence of agglutinins or other adaptive substances.
2. That the presence of these substances in very active sera may be demonstrated, as was first shown by Neufeld, by the usual agglutination methods, but that as a delicate or practical routine procedure this method was a failure.
3. That by concentrating the pneumococcus cells in an isotonic solution, as by centrifugalizing broth cultures and shaking the sediment with a small quantity of salt solution, a practical and accurate method is available for the precise study of the pneumococcus agglutination reaction.
4. That by this method an agglutinative action, heretofore not detected, has been demonstrated in the blood serum of various normal animals and in certain specifically immune sera.
5. That it has been possible to obtain the pneumococcus precipitin reaction, not only with cultures cleared by normal bile, as did Neufeld, but with filtered salt solution extracts of the pneumococcus cells.
6. Finally, that precipitin reactions, heretofore not detected, have been demonstrated with the blood serum of various normal animals and with certain immune sera.

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A PATHOLOGY FOR FORAGE POISONING, OR THE SO-CALLED
EPIZOÖTIC CEREBRO-SPINAL MENINGITIS OF HORSES.¹

(A PRELIMINARY REPORT.)

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The disease known as infectious or epizoötic cerebro-spinal meningitis of horses is but little understood. In all outbreaks there seems to be a common cause, and there is little or no evidence that the disease is ever transmitted from one horse to another. In some cases the origin can clearly be traced to the food, and Dr. Leonard Pearson has produced the disease by feeding ensilage taken from a stable in which animals had been attacked. The influence of food is well illustrated by an outbreak which occurred in a large stable in Philadelphia. It began in December, 1901, twenty-seven horses being affected, of which ten died. A fresh supply of food was obtained, and piled on top of the old. No new cases occurred under the use of this feed, but in May, 1902, the old food was again reached, and soon after fifty-nine horses developed the disease, twenty-four of which died, and six were destroyed. From his experiments and observations Dr. Pearson has proposed the name "forage poisoning," a name which is more in accordance with the facts as we know them at present. The term "cerebro-spinal meningitis" is not justified by the clinical history nor by post-mortem findings.

While forage is no doubt responsible for many of the outbreaks, the actual pathogenic agent has not yet been discovered, though a toxic mold or fungus is supposed to be the cause. All attempts to find a specific micro-organism in the animals affected have failed completely, nor has microscopic examination of the tissues revealed any specific lesion. Gross examination usually shows hyperemia of the brain and cord, and their meninges, with increase of fluid in

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the subarachnoid spaces and ventricles. This fluid is clear, and we have been unable to discover any micro-organism in it by cultural methods.

Symptoms.¹—The symptoms are referable to the central nervous system. In mild attacks there is loss of control over the limbs and tail, loss of appetite, and difficulty in swallowing. The inability to swallow is often a marked symptom in more severe cases, and the name "putrid sore throat" has been applied to the disease. There is stupor, apathy, extreme muscular weakness, or actual paralysis. A common symptom is contraction of the muscles of the neck, back, and loins, with more or less opisthotonos. Paroxysms of delirium occur, during which the animal will push against the wall, or show the disorderly movements due to meningeal irritation. Coma and paralysis come on, and death occurs in from five to forty-eight hours. In the most acute cases the animal falls and dies in convulsions.

It seems probable that several diseases which are characterized by similar clinical symptoms have been considered as one and the same by observers.

MacCallum and Buckley have found in the brains of horses dying of this disease areas of softening "in the frontal region on each side, anterior to the motor region of the cortex." This softening was practically confined to the white matter immediately under the cortex, the rest of the brain showing no abnormality. In these areas there was "complete destruction of the brain substance in which the anatomical elements are disintegrated, and largely replaced by a colloid-like material." The neighboring blood vessels were acutely inflamed, with exudation of leucocytes, and passage of the red corpuscles into the peri-vascular lymph sheath and adjacent tissues. In a second outbreak they failed to find the softened areas in the brain, but the condition of the blood vessels was such as to make them believe that they had the earlier stages of the same process.

¹ Moore. Pathology of the infectious diseases of animals.

They have given the name "Acute Epizoötic Leucoencephalitis." (Bulletin 80 of the Maryland Agricultural Experiment Station.)

The disease has engaged our attention at the laboratory of the State Live Stock Sanitary Board for several years, and examination by cultural methods have been made whenever possible, but always without result. We were led to the present investigation more than a year ago while making a study of the value of the rapid diagnosis of rabies after the method of Van Gehuchten and Nélis, in the course of which several horses and two calves, which had died of forage poisoning, were used as controls.

Pathological report. — With the exception of the lesions in the upper gastro-intestinal tract where the infection probably occurs, the only others discovered were confined to the central nervous system, and may be grouped for purposes of description as follows:

1. Lesions of the intervertebral and Gasserian ganglia.
2. Lesions in the cerebral and cerebellar cortex.
3. Lesions in the choroid plexuses of the lateral cerebral ventricles.
4. Lesions of the peripheral nerves supplying the larynx.

Fifteen animals have been studied. In the first six of these the intervertebral ganglia were not examined. In all the nine cases in which these structures have been studied the following changes have been found: In the normal ganglion the ganglion cells are enclosed in a capsule fitting closely around the cell. This capsule is made up of a single layer of endothelial cells. The supporting structure of the ganglion is composed of a loose areola of connective tissue, through which run the nerve fibers on their way to the spinal cord. All of these structures are affected.

The ganglion cells were the seat of extensive chromatolysis. The degenerative changes vary from a simple diffuse chromatolysis—a fusing together and loss of outline of the

fine chromatin points in the cell protoplasm—to complete destruction of the cell body and nucleus (Figs. 1 and 2). At times cells were found apparently normal, except for the accumulation of large amounts of a yellow pigment, staining black with osmic acid. In other cells, besides the diffuse chromatolysis above referred to, the nucleus was found displaced to the periphery of the cell. As the degenerative changes advanced, the cell protoplasm took the stain very strongly and appeared a deep blue by the Nissl method. Marked vacuolation of the cell protoplasm was present in two cases (Fig. 3). In four cases some of the ganglion cells were completely disintegrated, filaments of protoplasm remaining among the small mononuclear cells surrounding the capsule.

Capsular and pericapsular changes. — In all nine cases in which the intervertebral ganglia were examined a peri-capsular, small round cell accumulation was present (Fig. 2). In some of the degenerating ganglion cells a few nuclei were seen within the capsule in the degenerating cell protoplasm. The accumulation of nuclei around the cell capsule did not always assume a concentric shape, but was often eccentric extending irregularly into the stroma. The cells are all of the small type, the nuclei and protoplasm being about the size of a red blood corpuscle. There is no evidence that these cells are due to a proliferation of the original layer of capsular cells. Polynuclear cells, or cells with an irregular nucleus, were not present in any of the specimens examined. It is probable, inasmuch as these cells stand in no relation to the vessels of the ganglia, that they are the result of a proliferation of the stroma cells of the ganglion.

Cortical lesions. — The cortex of the cerebrum and cerebellum was markedly congested both to gross and microscopic examination. The meninges were normal. The ganglion cells were normal to the Nissl and other cell stains. Numerous capillary hemorrhages were scattered throughout the entire cortex of the cerebrum and cerebellum. There were also hemorrhages in the subcortical tissues. The basal

ganglia, pons, and medulla were perfectly normal. The spinal cord, outside of some congestion of the gray matter, was normal. The meninges showed no trace of an inflammatory process.

Lesions of the choroid plexus. — The choroid plexus in three of the cases was changed from a filmy membrane to a large triangular tumor-like mass. This mass was of a yellowish-red color, of firm consistency, and measured two and a half centimeters in transverse section. On microscopic examination the increase in size was found to be the result of a proliferation of the elastic tissue surrounding the vessels. By the Van Giesen stain the entire section was found to consist of whorls of delicate fibers starting from the neighborhood of the vessel walls and extending to the margin of the plexus (Fig. 4). These fibers were not nucleated, although numerous nuclei of the supporting tissue of the gland were present between the whorls. At the suggestion of Dr. Flexner, the Weigert elastic stain was used and the character of the tissue determined. The ependymal cells covering the villi were normal.

The peripheral nerves. — An examination of the nerves supplying the larynx and the neck by the fresh osmic acid method showed a slight but distinct degeneration. This was present in the nerve up to the ganglion, but was not present in the posterior roots, or the root of the fifth nerve. These lesions in the myelin corresponded to the presence of a marked degree of swelling of the axis cylinder in the substance of the ganglion. Hemorrhagic extravasation into the sheath of the pneumo-gastric nerve was present in one case.

Summary.—Hemorrhagic inflammation of the upper respiratory organs; degeneration of the peripheral nerves supplying these areas; toxic irritation of the intervertebral ganglion as manifested by intense degeneration of the ganglion cells, pericapsular round cell infiltration, and swelling up of the axis cylinders; widespread capillary hemorrhagic extravasation

of the cortical and sub-cortical tissues, tumor formation due to proliferation of elastic tissue of the choroid plexus of the lateral ventricles.

The ganglionic lesions above described closely resemble those described by Van Gehuchten and Nélis in rabies. In rabies, however, there is an active proliferation of the capsular cells with a marked tendency to extension within the capsule, while, as has already been pointed out, the tendency in this disease is to a pericapsular accumulation of cells. In advanced cases of forage poisoning the ganglion cells may entirely disappear and an accumulation of small round cells remain. Under these circumstances the picture cannot be differentiated from rabies by an examination of the ganglion alone. The perivascular round cell accumulation in the pons and medulla, which is rather constant in rabies, is never present in forage poisoning. There is no degeneration of the peripheral nerves in rabies. The clinical course of the two diseases is entirely different, and there should be no difficulty in separating the two conditions by the pathological lesions.

Professor Van Gehuchten, of Louvain, to whom we submitted the specimens from our first case (a calf), confirmed our opinion that there was a distinctive difference between the ganglionic changes in forage poisoning and in rabies.

Concerning the specimen sent to him he writes: "It cannot be denied that there is a sensible proliferation of the cells of the endothelial capsule, but this proliferation does not, however, appear to me to be as intense as in cases of rabies; so much so, that I would not make the diagnosis of rabies from the examination of the sections alone. I do not think that this animal had rabies. Rabies excluded, there remains a certain amount of proliferation, the cause of which escapes me; but in my opinion the degree of proliferation cannot be compared with that which occurs in rabies."

CONCLUSIONS.

1. The so-called epidemic cerebro-spinal meningitis of horses is not a true meningitis, and presents neither the gross nor microscopic lesions of true meningitis.

2. The evidence goes to show that all epidemics are caused by some poisonous substance contained in the forage. This is proven conclusively in the epidemic mentioned above, and in the experiments of Dr. Pearson.

3. The lesions in the intervertebral ganglia so closely resemble those described by Van Gehuchten and Nélis in rabies, as to offer the presumption that the pathological process in the two diseases is somewhat similar.

4. The differential diagnosis between forage poisoning and rabies depends upon (*a*) the absence from the medulla and pons in forage poisoning of the perivascular and peri-cellular lesions (Rabic tubercles of Babes); (*b*) in forage poisoning there is predominance of peri-capsular rather than intra-capsular round cell infiltration of the ganglion cells. (*c*) Lesions of the larynx and laryngeal nerves. The clinical history is always conclusive.

5. Forage poisoning is a much better and more comprehensive term than "cerebro-spinal meningitis," or than "leucoencephalitis," as proposed by MacCallum and Buckley.

EXPLANATION OF PLATE II.

FIG. 1. — Intervertebral gland of horse. Forage poisoning. x 210.

FIG. 2. — Gasserian ganglion. Cellular degeneration with peri-capsular round-cell infiltration. Forage poisoning. x 210.

FIG. 3. — Gasserian ganglion of horse. Forage poisoning. x 210.

FIG. 4. — Choroid plexus, occurring in two cases of forage poisoning. Van Giesen staining. x 210.

CAT'S BLOOD — DIFFERENTIAL COUNTS OF THE LEUCOCYTES.

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Differential counts of the leucocytes in the blood of twenty normal cats were made to determine, if possible, a normal standard for this animal. Counts of the red and white corpuscles were also made. The staining methods upon which the percentages in the accompanying table are based are those of Leishman and Wright's modification of the same. In order to check results, smears were also stained with Ehrlich's triacid mixture, with methylene blue, with polychrome methylene blue, and with Dahlia. Blood was taken twelve hours or longer after feeding.

Morphology and staining reactions of the various leucocytes. — The leucocytes of cat's blood may be divided, according to the form of the nucleus, into polymorphonuclear and mononuclear cells.

The polymorphonuclear may be sub-divided into granular and non-granular forms. The granular forms are distinguishable into those with large coarse granules, those with medium coarse granules, and those with fine granules. The fine granular forms are comparatively few and probably belong in the same class with the non-granular polymorphonuclear form which is the most frequent in the cat's blood.

The mononuclear forms may be differentiated into a small mononuclear (lymphocyte) and a large mononuclear. The large mononuclear may be further differentiated, according to the nucleus, into one with a bean or kidney shaped nucleus and one with a spherical nucleus.

The non-granular polymorphonuclear. — This form varies in size from a cell of a diameter only slightly greater than that of a red blood corpuscle to one of three or four times

that length. The cytoplasm is apparently homogeneous, taking a very faint blue or pink hue. The nucleus is polymorphous, having the appearance of a twist or knot, and with Wright's method stains a lilac shade. It corresponds in size and appearance to the polymorphonuclear cell of dog's blood.

The fine granular polymorphonuclear. — This cell is similar to the non-granular form, except that the cytoplasm contains a variable number of very fine granules having a faint affinity for eosin. These granules may be few and faintly stained, or the cytoplasm may be packed with granules taking quite a definite dark red stain. Other cells are seen with merely a granular appearance of the cytoplasm, but no definite granules. They are probably to be classed with the non-granular polymorphonuclear cells.

Coarse granular forms, oxyphile cells.¹ — These cells show a great variety in size and in the number, size, and form of the granules. There are two extremes with numbers of intermediate forms, which has made it difficult to classify them separately. We have, however, in our differential counts, under the heading of coarse granular oxyphile cells, differentiated two forms according to the size and shape of the granules. The granules all stain more or less intensely with eosin and vary in shape from spherical to that of rods with round ends. Occasionally a cell is seen as large as the large mononuclear forms, with long, narrow, rod-like granules having the appearance of bacilli. These cells frequently appear as a nucleus, with a number of free rods about it as if the cell body had burst and discharged its contents. The majority of the coarse granular oxyphiles are of about the same size and shape of granules as those found in the dog. There are cells, however, which are but little larger than a lymphocyte, where the granules are smaller and closely packed. These may possibly correspond to the cells which Hirschfeld²

¹ Classification of Kanthack and Hardy. *Journal of Physiology*, xvii, p. 81.

² H. Hirschfeld. *Arch. f. Path. Anat. u. Physiol.*, Bd. 149, Hft. 1, July 5, 1897.

describes as neutrophiles, because of their tinctorial qualities with Ehrlich's triacid mixture.

The nucleus, as a rule, is of the same form as that of the other polymorphonuclear cells. In the larger granular cells the nucleus often consists of two apparently unconnected bodies situated at opposite poles of the cell. The nucleus stains less intensely than that of the non-granular polymorphonuclear cell, and takes more of a blue shade. Mast cells were only occasionally seen in the blood of cats which we examined.

The mononuclear forms.—The small mononuclear or lymphocyte is slightly larger than a red blood corpuscle, and consists of a round nucleus surrounded by a narrow rim of cytoplasm. The nucleus stains a dark lilac, and the cytoplasm a faint blue. The chromatin has in most cases a typical mural arrangement. Frequently cells are seen with a nucleus apparently undergoing division.

The large mononuclear leucocyte has a diameter from two to three times that of the small mononuclear form.

The cytoplasm is relatively greater in proportion to the nucleus than in the lymphocyte. The nucleus may be either circular or indented. There is a tendency toward mural arrangement of chromatin. The nucleus takes a lilac stain; the cytoplasm a faint blue. The nucleus does not stain so deeply, however, as does that of the small mononuclear form, while the cytoplasm stains somewhat more deeply.

TABLE OF DIFFERENTIAL COUNTS.

NUMBER OF CAT.	POLYMORPHONUCLEAR CELLS, PER CENT.				MONONUCLEAR CELLS, PER CENT.		
	Coarse granular.		Fine granular.	Non- granular.	Large.	Small.	Mast cells.
	Large Oxy- philes.	Medium Oxy- philes.	Oxy- philes.				
I.....	1.40	3.35	2.24	56.84	7.28	28.89	0.00
II.....	0.63	5.00	1.60	50.00	6.50	36.27	0.00
III.....	3.96	5.94	2.80	47.52	10.62	28.98	0.18
IV.....	1.00	2.34	2.52	49.86	9.36	34.92	0.00
V.....	1.96	7.84	2.62	49.54	8.82	29.22	0.00
VI.....	0.30	6.00	1.80	58.20	5.30	28.30	0.10
VII.....	1.20	2.80	1.80	50.60	4.20	39.40	0.00
VIII.....	0.40	3.10	0.40	64.00	1.70	30.40	0.00
XI.....	0.25	2.25	2.50	65.25	5.25	24.25	0.25
XII.....	0.44	2.49	0.44	53.59	4.84	38.20	0.00
XIII.....	2.10	8.90	1.10	44.10	3.70	41.1	0.00
XIV.....	1.20	5.30	2.50	53.50	4.60	32.90	0.00
XV.....	0.20	2.80	0.00	66.00	2.40	28.60	0.00
XVI.....	0.30	5.70	0.60	60.20	3.00	30.20	0.00
XVIII.....	0.20	5.30	0.70	59.50	5.80	28.50	0.00
XIX.....	0.68	3.82	0.30	53.90	3.20	38.10	0.00
XX.....	0.93	5.67	1.70	55.00	3.40	33.30	0.00
XXI.....	0.561	2.992	0.561	48.246	3.553	43.90	0.187
XXII.....	0.200	3.100	0.300	46.70	1.8	47.90	0.00
XXIII.....	0.300	2.8	0.600	49.30	2.6	44.40	0.00
Average.....	0.90555	4.3596	1.36405	54.1523	4.89615	34.3865	0.03585

The minimum number of red corpuscles per cubic millimeter of blood was 4,805,000; the maximum was 7,610,000; the average was 6,609,000.

The minimum leucocyte count was 7,219; the maximum, 19,000; the average, 13,331.

SUMMARY.

1. In circulating cat's blood seven varieties of leucocytes may be distinguished. These may be divided into polynuclear forms and mononuclear forms, also into granular and nongranular forms. The latter are the more numerous.

2. There are three polymorphonuclear forms with oxyphile granules: a large coarse granular form with rod-shaped granules (Fig. 7), a smaller, coarse granular form with round granules (Fig. 6), and a fine granular form (Fig. 5). Between these three there are a number of transition forms.

3. The mononuclear cells are of two kinds, a large and a small, the former with considerable cytoplasm and with either a bean-shaped or spherical nucleus (Fig. 3), the latter with a spherical nucleus and a narrow rim of cytoplasm (Fig. 2).

4. Mast cells may be present, but in small numbers.

5. The approximate percentages of the various forms is as follows: Polymorphonuclear without granules, 54.1523 (Fig. 4); with large coarse oxyphile granules, 0.90555; with medium coarse oxyphile granules, 4.3596; with fine oxyphile granules, 1.36405; large mononuclear forms, 4.89165; small mononuclear forms, 34.3865; mast cells, 0.03585.

EXPLANATION OF PLATE III.

FIG. 1. — Red blood corpuscles for comparison.

FIG. 2. — Small mononuclear leucocyte.

FIG. 3. — Large mononuclear leucocyte.

FIG. 4. — Nongranular polymorphonuclear leucocyte.

FIG. 5. — Fine granular oxyphile.

FIG. 6. — Medium coarse granular oxyphile.

FIG. 7. — Large coarse granular oxyphile.

THE REACTIONS OF THE BLOOD IN EXPERIMENTAL DIABETES MELLITUS. — A CONTRIBUTION TO OUR KNOWLEDGE OF THE THERMOLABILE COMPLEMENTS.*

First Contribution.

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Our work upon experimental diabetes mellitus was suggested by the fact, long known to clinicians and to students of immunity, that the diabetic organism is abnormally susceptible to infectious processes. The important advances of the last few years in our knowledge of the mechanism of immunity make possible an analysis of such a phenomenon as that offered in diabetes, and suggest that the disease may present conditions through the study of which further knowledge may be obtained of some, at least, of the undecided problems which still confront us. Until these additions to our knowledge were made, only the fluctuations in the bactericidal activity of a serum could be recorded. Our present methods enable us to determine which of the two factors concerned in bacteriolysis or hemolysis is principally influenced by the abnormal process. It is also possible that the loss of resistance to infection is due to a change in the receptivity of the cells of the organism to the bacterial toxins. This side of the problem may be approached by experiments in vitro; *i.e.*, by a comparative study of the effect of specific toxins upon suspensions in the test-tube of the cells of the normal and the diseased organism.

One might suspect, a priori, that the decreased resistance of the diabetic is due to a loss of complements, since the complements seem to be the least stable of the three factors concerned in immunity, — amboceptor, complement,

* Read before the American Association of Pathologists and Bacteriologists, May, 1903. Received for publication June 24, 1903.

receptor, — yet a suspicion remains such until the suspected fact is proven, and a priori assumptions do not necessarily hold good. The remaining possibility would of itself have justified the problem — the possibility of obtaining some light upon the disputed questions of the source and the nature of the thermolabile substances known as complements.

In the course of this presentation we shall not enter at all extensively into a discussion of the vast literature of the different forms of diabetes which we have included in our work. It has been established beyond question that certain interferences with the animal organism will set up a more or less intense excretion of glucose, which may vary in amount from a simple glycosuria to a true diabetes. We shall start from the facts that phlorizin, adrenalin chloride, and the complete removal of the pancreas cause glycosuria and diabetes, and shall present our results, drawing from the literature only where it is necessary to complete and confirm our work.* It seems to us utterly futile to attempt at present any explanation of the facts which we shall present; still more futile would it be to enter here upon a discussion of the similarities and quantitative differences in the metabolic processes which characterize the forms of diabetes which we shall consider.

We have directed our attention mainly to the question of hemolysis, because we believe that the methods employed in the study of the phenomena of hemolysis alone permit of an exact quantitative expression of what is taking place. So long as we have no practical method for obtaining a bacteriolytic complement free from its pairing amboceptor, so long must we argue from analogy with the hemolytic process, when we come to the ultimate analysis. To be sure, a number of workers have recently reported experiments in which the complement-content of a given serum was studied by

* We have also considered the possibility of rendering the pancreas functionally inactive by means of the serum of an animal which had been immunized against the cells of the pancreas of another species. Some preliminary work with the serum of a duck treated with injections of rabbit's pancreas did not seem to hold out the least promise of success. We were further discouraged from this attempt by the negative results with a specific serum for the adrenal glands which were recently reported by Abbott.¹

"reactivating" a heated serum with certain amounts of the fresh serum in question. We have never been able to understand such a procedure, for the reason that the fresh serum used for the "reactivation" has, in every case, contained both the bacteriolytic amboceptors and the bacteriolytic complements. Now we know that every serum, normal as well as specific immune serum, contains amboceptors in excess of the complements present. Why, then, add a quantity of amboceptors when the fresh serum already contains more than enough amboceptors to satisfy the complements which it contains? The only result can be an "Ablenkung" of the complements, in the sense of the phenomenon described by Neisser and Wechsberg. Further, it has been shown in the work of Vedder² in this laboratory that isotonic salt solution can be "reactivated" in precisely the same way as a heated serum, by the addition of very small amounts of a fresh serum. With these theoretical considerations and practical results before us, there was evidently no object in our performing any such experiments. We have included bactericidal experiments in our work, and shall present the results, but they are not entirely satisfactory because they are not analytic.

I.

PHLORIZIN DIABETES.

We first turned our attention to the study of phlorizin diabetes for two reasons. Leo,³ as long ago as 1889, reported that white mice lost their natural immunity to the glanders bacillus when they were fed with phlorizin. In the second place, phlorizin diabetes offers a necessary control to the work with true diabetes, since there occurs as marked an hyperglycemia as in diabetes proper, but without the involvement of the organs which is necessary to a true diabetes; we were further interested in studying the effect upon the blood reactions of a process in which the kidney is, perhaps, principally involved.

Rabbits were chosen for these experiments; the serum of the normal rabbit contains no amboceptor for bovine erythrocytes. If, therefore, we use a specific active serum,

obtained by immunizing rabbits against bovine erythrocytes, heat the same, and reactivate with normal rabbit serum, we have a combination in which the fresh serum represents an addition of so-called "pure" complement; *i.e.*, a complement free from the pairing amboceptors. The effect of the phlorizin upon the immune body was studied by treating with the glucoside a group of animals immunized against bovine erythrocytes.

The phlorizin was administered to the animals by subcutaneous injections of a concentrated solution of phlorizin in fifty per cent alcohol. The urine was collected in especially constructed cages, arranged with a funnel-shaped, metal bottom, which conducted it into a beaker placed beneath; above this bottom is a perforated screen, strong enough to support the animal, and with openings small enough to prevent the passage of the feces. This is doubtless not an ideal method for fine determinations of metabolic processes, offering, as it does, the opportunity for contamination of the urine with the fecal matter, and disregarding the possible loss of sugar by fermentative processes. It is, however, sufficiently accurate for our purpose, and is a thoroughly practical method. The urine was collected at about the same hour each day, and examined by means of the Fehling reduction method, the fermentation test, and in doubtful instances by means of the polarimeter.

The general details of the experiments are as follows: A group of three normal rabbits was chosen, and placed for a day or two in the cages until examination could assure us that the animals were perfectly healthy, in the matter of the excretion of glucose, at least. The animals were then bled, and the complement-content of their sera tested in the manner to be described later. Two of the three rabbits were then subjected to the treatment with phlorizin, and after the completion of the treatment all three rabbits were again bled and the sera tested in exactly the same manner as that used to test the sera obtained at the first bleeding. In this way the results were controlled doubly, once by the first bleeding,

and again by the result with the serum of the normal rabbit included in the experiment group.

The specific immune serum was obtained from rabbits highly immunized by successive intraperitoneal injections of defibrinated bovine blood. The corpuscles used in the hemolytic series were thoroughly washed, five cubic centimeters being washed in two changes of 0.85 per cent sodium chloride solution, and finally suspended in one hundred cubic centimeters of fresh 0.85 per cent sodium chloride solution. For the sake of brevity we shall not include the records of all the experiments, but will give the results alone, and will present one complete protocol as an example of the manner in which all these experiments were conducted.

Effect of the subcutaneous injection of phlorizin upon the complement-content of the serum of normal rabbits.

October 29, 1902 :

Three normal rabbits, A, B, and C, the urine from which contained no sugar, were bled by means of Latapie apparatus, and the serum collected. The following day the sera were tested as follows :

To each tube of three parallel series of nine tubes in each series, each tube containing 1.0 cc. of a 5 per cent. suspension in 0.85 per cent. NaCl solution of washed bovine erythrocytes, was first added 0.05 cc. of heated immune serum from a rabbit immunized against bovine blood.

To Series I. was added the serum of rabbit A in the following amounts : 0.002, — 0.004, — 0.006, — 0.008, — 0.01, — 0.02, — 0.03, — 0.04, — 0.05 cc. To Series II. were added exactly the same amounts of serum B. To Series III. were added exactly the same amounts of serum C.

After two hours at 37° C., and over night in the ice-box, all three series exhibit exactly the same degrees of hemolysis ; hemolysis begins in the tubes containing 0.002 cc. of the serum A, B, or C, and increases regularly in all three series to almost complete hemolysis in the tubes containing 0.05 cc. of the serum A, B, or C. Control tubes, made by adding 0.1 cc. of the serum A, B, or C alone, or of 0.1 cc. of the heated immune serum alone, to tubes containing 1.0 cc. of the blood suspension, showed no hemolysis.

November 3, 1903 :

The three rabbits, A, B, and C, were bled a second time, and the sera were collected. Rabbits A and B had each received a total of six grams of phlorizin, given in three subcutaneous injections ; the urines from A and B had contained considerable amounts of glucose, as shown by the fermentation test, during the time of the injections. The excretion of

glucose had ceased as soon as the injections of the glucoside were discontinued. Rabbit C had been kept under the same conditions of food and confinement, but had not been subjected to any treatment whatever. The following day the complement-content of the sera was tested in the same manner as at the first bleeding, the same heated immune serum being used.

After two hours at 37° C., and over night in the ice-box, the series show the following results: Hemolysis in the series made with sera A and B is alike; hemolysis begins in both series in the tubes containing 0.002 cc. of the serum A or B, and increases regularly to complete hemolysis in the tubes containing 0.03 cc. of the sera. The control series made with the serum of rabbit C shows beginning hemolysis in the tube containing 0.002 cc. of serum C; hemolysis increases regularly with the series to complete hemolysis in the tube containing 0.04 cc.

Control tubes show no hemolysis.

It will be observed that the hemolytic action of the serum of the control rabbit C seems also to have slightly increased in the second series. To what this is referable is not clear, but it is evident that all the conditions of such an experiment cannot be kept exactly identical. We have endeavored to keep the conditions as nearly alike as possible, and believe that the use of the same immune serum in both initial and final experiment, and the observance of constant temperatures have assured us approximately identical conditions. But we have not been able to secure the bovine blood from the same animal for the successive experiments, having obtained the blood at the abattoir, and the individual difference in the susceptibility of the erythrocytes from different animals may account for such slight differences as we see in this experiment.

It appears from the above experiments that the serum of both rabbits which had been treated with subcutaneous injections of a total of six grams of phlorizin in alcoholic solution shows a slight increase of hemolytic complement. A similar experiment was tried with three rabbits; one received subcutaneous injections of phlorizin, one was given phlorizin by means of the stomach tube, and the third was left without treatment as control. The animal treated with a total of ten and one-half grams of phlorizin by means of the stomach tube showed no excretion of glucose, also no change in the

complement-content of its serum. The animal treated with subcutaneous injections of a total of ten grams of phlorizin showed an increase of the hemolytic complement. The amounts of sugar excreted by this animal are shown in the following table, which also illustrates the manner, in which the experiments were conducted :

TABLE I. — PHLORIZIN RABBIT F.

Date.	Weight.	Amount in cc.	Ferment.	Fehling in per cent.	Total sugar.	Treatment.
Nov. 17	1,650	245	Negat.			
" 18	1,700	260	"	Bled, then injected with 2 grams phlorizin.
" 19	1,700	175	Posit.	2.25	3.94 g.	2 grams phlorizin, in 50 per cent alcohol.
" 20	1,670	295	"	2.25	6.64	2 grams phlorizin, in 50 per cent alcohol.
" 21	1,620	215	"	2.94	6.32	2 grams phlorizin, in 50 per cent alcohol.
" 22	1,620	295	"	1.25	3.69	2 grams phlorizin, in 50 per cent alcohol.
" 23	1,540	320	"	2.08	6.66	No treatment.
" 24	1,540	165	"	2.5	4.12	Bled.
" 25	1,520	175	Negat.	0.45	0.79	
" 26	1,470	170	"	Negat.		

In a further experiment three rabbits which had been highly immunized against bovine erythrocytes were chosen, and two of them were subjected to treatment with subcutaneous injections of phlorizin. The comparison of the hemolytic action of the heated serum of these rabbits, reactivated with normal rabbit serum, showed no change in the content of specific amboceptors.

It may be objected to these experiments that the treatment with the phlorizin was not continued for a sufficient length of time to cause any changes in the serum reactions. We grant this point, even though it will appear from our later work with pancreatic diabetes that a more extensive process is probably necessary before the normal equilibrium between amboceptor and complement is disturbed. It was

impossible to continue the treatment for a longer period, for the reason that the subcutaneous injection of phlorizin causes extensive local inflammatory processes, which are due to the irritation of the solution used for the injection, and to the presence of the hard mass of phlorizin left behind at the point of injection after the absorption of the alcohol. It has been conclusively shown that the inflammatory process is accompanied by an increase of complements in the serum (see ² and ⁴). The result of our work with phlorizin is, therefore, not surprising, — the slight increase of hemolytic complement is to be explained as occurring coincident with the inflammatory process.

II.

ADRENALIN GLYCOSURIA.

Our attention was next directed to the study of the glycosuria caused by the intraperitoneal injection of adrenalin chloride. But here we immediately encountered the difficulty met with by all workers with the specific extract of the adrenals, — the impossibility of determining the minimum lethal dose. Our results were identical with those reported by Abbott.¹ An injection of one cubic centimeter of adrenalin chloride proved fatal to some of our rabbits, while others showed no remarkable effects after successive injections into the peritoneum of two cubic centimeters. One dog showed only the usual transitory effects after three injections of four, five, and six cubic centimeters; two dogs were killed by one injection of three cubic centimeters; one dog, weighing twenty-seven pounds, died after one injection of three cubic centimeters, while another dog, weighing twenty-one pounds, bore the injection of four cubic centimeters.

Of two rabbits treated with daily injections of one and two cubic centimeters of adrenalin chloride, one showed an irregular excretion of glucose, while the other rabbit excreted no sugar during the whole course of the experiment. The urines were tested by the Fehling and fermentation tests, and also by the polarimeter; the highest percentage

of glucose in the urine of the one rabbit was .63 per cent, equivalent to a total of six and three-tenths grams for the twenty-four hours. These animals were not bled before the treatment, for the reason that the high mortality makes such a procedure seem futile; the control test was therefore made with the sera from two normal rabbits. The two rabbits treated with adrenalin chloride in this experiment received a total of twenty-one and twenty-two cubic centimeters respectively of the adrenalin. The complement-content of their sera was tested in the same manner as in the experiments with phlorizin, by reactivating a specific immune serum for bovine erythrocytes. The serum of neither of these animals showed the slightest deviation from the reactivating power of the sera of two normal rabbits, tested under the same conditions.

The serum of a normal dog was tested upon five per cent suspensions of washed guinea-pig and of washed rabbit corpuscles. It was found that 0.1 cc. of the serum of this dog caused the complete hemolysis of the corpuscles contained in one cubic centimeter of the five per cent suspension of guinea-pig's corpuscles; complete hemolysis of one cubic centimeter of rabbit's corpuscles was produced by 0.08 cc. of this serum. The dog was then given three injections into the peritoneal cavity of four, five, and six cubic centimeters of adrenalin chloride, and the serum again tested. It was now found that 0.06 cc. of the serum sufficed to cause complete hemolysis of one cubic centimeter of guinea-pig's corpuscles, and that 0.07 cc. was necessary to completely hemolyze rabbit's corpuscles. There seems to have been a slight increase of hemolytic activity.

We recognize fully that our experiments with both phlorizin and adrenalin chloride are not numerous enough to warrant drawing conclusions from them alone; but the indications offered did not seem to justify the further expenditure of the great amount of time necessary for such experiments. These few results, taken in connection with the work upon true diabetes which we shall present later, seem to us, however, to justify the conclusion that a glycosuria or a glycemia have of themselves no influence upon

the state of immunity. The fact that the complete removal of the pancreas, in other words, the induction of a true diabetes with the consequent effect upon the animal metabolism, is necessary to produce a loss of complements, substantiates the evidence offered in these few experiments, that such a transitory process as phlorizin and adrenalin glycosurias can have no action in lowering the resistance of the organism.

III.

TRUE DIABETES MELLITUS.

Although we are aware of the many points of similarity between the metabolic processes in phlorizin and adrenalin glycosurias and in true diabetes, we do not, from the standpoint of our own work, consider them at all comparable, for the reason that neither phlorizin nor adrenalin causes those profound disturbances the expression of which is a constant excretion of glucose. In both instances the sugar disappears from the urine very soon after the treatment is discontinued; and it is evident that such a transitory process cannot affect the blood reactions, else we should expect a phenomenon like that seen in severe diabetes mellitus—a loss of resistance—in many minor diseases which are accompanied by glycosuria. A glycosuria or a glycemia have of themselves no effect upon the serum reactions; but with the glycosuria and the glycemia of a true diabetes mellitus there occur those profound metabolic disturbances which lead to the ultimate disappearance of one of the factors concerned in the normal immunity of the organism to infection.

We therefore took up the study of a true diabetes mellitus. It is known since the work of v. Mering and Minkowski⁵ that a true experimental diabetes mellitus, characterized by a marked and progressive loss of body weight, great increase of hunger and thirst, polyuria with the constant excretion of large quantities of glucose, muscular weakness and final death in diabetic coma, can be produced in animals by the complete removal of the pancreas. This operation has been performed upon a large number of animals, but the dog has been most often used; among other reasons, the anatomy of

the dog's pancreas permits the complete removal of the organ without the complication of the resection of the intestine. It was mainly for this reason that the dog was chosen for our work. This animal offers another decided advantage in such a problem; the serum of the normal dog contains considerable amounts of the amboceptors as well as the pairing complements for the erythrocytes of a number of our common laboratory animals, and thus affords the opportunity for a study of both amboceptor and complement in the serum of the same animal.

The urine from the diabetic dogs was collected in cages constructed upon the same principle as those used for the collection of the urine from the rabbits under treatment with phlorizin and adrenalin. Such cages offer the same advantages and disadvantages as those used for the rabbits, but have proven themselves to be of practical value. The urine was collected at about the same hour each day and examined by the Fehling and fermentation methods; the specific gravity and the total amount excreted were also recorded. The dogs were fed with milk, bread, and meat; accurate record of the amounts fed was not kept, since such record did not seem to us to be of any value in the work. If we were dealing with a process which fluctuates from day to day, or which is susceptible to transitory changes in the metabolic processes, such a record might have been of value. In general, however, we may state that the dogs received about one-half liter of milk, about sixty grams of bread, and from five hundred to two thousand grams of meat per day, the amount of meat varying with the increasing appetite of the animal.

The operation for the complete removal of the pancreas has been performed in various ways. We have preferred to extirpate the entire organ at one operation, following in general the technical details described by Minkowski.⁶ The operation is not a difficult one. We have operated upon fourteen dogs, the autopsy showing that the pancreas was completely removed, except for the occurrence of aberrant islands of pancreas tissue in two animals. These two animals will be discussed later. Of the remaining twelve

dogs, three died of general peritonitis,—No. IX. after two days, No. XVII. after six days, and No. XVIII. after three days; No. VII. died of the effects of intestinal necrosis; No. X. died after two days, the autopsy not revealing a cause for death,—possibly to be ascribed to shock; No. XV. died after twenty-four hours, also no satisfactory explanation of death; No. XI. died after five days, No. XII. after seven days, and No. XIV. after four days, from infectious processes at the site of operation, of a more or less extended character. Of the remaining three dogs, two, No. VIII. and No. XIX., died after nineteen and twenty-one days respectively, in the typical condition of uncomplicated diabetes mellitus. Number XIII. died after twelve days from a purulent pleuritis. The urines from all these dogs contained glucose in sufficient amounts to warrant the belief that the pancreas had been entirely removed, except in the case of No. XX., which gave no excretion of glucose, and at autopsy no pancreas tissue could be found at the normal site of the organ.

The difficulty in such an experimental problem as ours has been to keep the animals alive for a sufficient length of time to permit of recovery from the immediate inflammatory processes which must unavoidably accompany the healing of the wound. In no instance of complete removal of the pancreas have we been able to secure primary healing. Because of the fact that suppurative processes are accompanied by an increase of the complementary substances, we have not examined the serum of the diabetic dogs until it was evident that no infectious process had supervened, and that the wound had healed as perfectly as is possible in a dog suffering from diabetes. The successive withdrawals of fifty cubic centimeters of blood from an animal so weakened by the disease process further complicates our problem by hastening the end.

The hemolytic properties of the serum of the diabetic dogs were tested upon the thoroughly washed erythrocytes of both the rabbit and the guinea-pig. The bactericidal properties of the serum were tested upon several varieties of

Staphylococcus pyogenes aureus, two of the strains having been isolated from dogs from which the pancreas had been removed; further, upon *Bacterium coli communis*, *B. typhi abdominalis*, and *B. dysenteriae* (Shiga). Both hemolytic and bacteriolytic tests were controlled by parallel tests with the serum of a normal dog, bled at the same time as the diabetic dog.

TABLE II.

Dog No. VIII.—Large black mongrel bitch, weighing about twenty-eight pounds. Pancreas removed Feb. 3, 1903.

Date.	Amount in cc.	Fehling in %.	Ferment.	Total sugar.	Treat- ment.	Notes.
Feb. 4	300	12.0	Posit.	36.0		
" 5	350	12.0	"	42.0		
" 6	290	12.0	"	34.8	Bled	
" 7	190	15.9	"	30.2		
" 8	285	9.6	"	25.8		
" 9	250	9.6	"	24.0		
" 10	225	14.5	"	32.6		
" 11	250	13.7	"	34.3	Bled	Appetite begins to improve.
" 12	340	15.9	"	54.0	Removed plaster cast, bandages, and stitches; wound united, but not very firmly; cast has chafed the hind legs and caused wounds which do not subsequently heal.
" 13	550	6.0	"	33.0		
" 14	370	8.0	"	29.6		
" 15	370	7.0	"	25.9		
" 16	200	9.6	"	19.2		
" 17	200	12.0	"	24.0	Dog eats well; stools contain great amounts of fat; very emaciated.
" 18	270	12.0	"	32.4		
" 19	300	13.7	"	41.1	Bled	
" 20	Beaker left from under cage, urine lost; marked polydipsia.
" 21	240	8.0	"	19.2		
" 22	150	12.0	"	18.0	Bled	Found dying, and dies while on the operating table.

Autopsy of dog No. VIII. — The wound of the skin has opened in two places, under which there is slight suppuration of the deeper layer of sutures. Peritoneum and intestines are normal. There is absolutely no fat in the body; the tissues are remarkably dry. Lymph glands of the mesentery are prominent, because of the absence of fat, but are not enlarged. The same is true of the retro-peritoneal lymph glands. Spleen and adrenals are normal; kidneys are of normal size, capsule not adherent, cortex shows slight fatty metamorphosis. Liver adherent to the site of the pancreas; on separating the adhesions two or three small foci of suppuration are found. The liver shows marked fatty metamorphosis; the center of the acini is of a yellow color, surrounded by a narrow ring of normal colored liver tissue. Heart and lungs appear normal. There seems to be very little blood in the body. No pancreas tissue is to be found.

The lymph glands, spleen, and adrenals of dog No. VIII. present a normal microscopic appearance. The liver sections show a marked fatty metamorphosis of the cells of the center of each acinus; for about two-thirds of the distance from the central vein to the periphery of each acinus the cells present a reticulated appearance, while the cells of the periphery show little or no evidence of degeneration. Under the high power the cells of the center of the acinus are seen to be made up of a normally stained nucleus and nucleolus; the cell protoplasm has almost entirely disappeared, what remains being in the form of a fine network. The meshes of this reticulum show that the fat was present in the form of relatively large droplets. The cells toward the periphery show under the high power evidence of slight fatty metamorphosis, while nearer the boundary of the acinus the liver cells have retained their normal appearance; in other words, there is no distinct line of demarcation between the infiltrated and the normal cells, but a gradual transition. The portion of oldest degeneration is evidently the center of the acinus. The reticulated cells are larger and more nearly round than normal; the nuclei show depressions and irregularities, as though caused by the pressure of the fat droplets. The glomeruli of the kidney are normal; the epithelial cells of the descending loops of Henle present a reticulated appearance, similar to that seen in the liver; the nuclei are normal, and are surrounded by the remains of the protoplasm in the form of a fine reticulum; the meshes of the network were evidently filled with droplets of a more uniform size than those in the liver cells. Otherwise the kidney presents a normal appearance.

Autopsy of dog No. XIX. — Dog dies a short time before we are prepared to bleed him. Body is cold, but the blood has not yet coagulated in the vessels. Thorax is opened, heart incised, and blood removed from the thoracic cavity with sterile pipette. At the site of the operation is a round wound, filled with granulation tissue. Slight suppuration beneath the skin, along the line of the deeper sutures. Peritoneum normal; site of pancreas adherent to the omentum and to a loop of the intestines; on pulling the adhesions apart, a small focus of suppuration appears. At the site of the pancreas is a small bit of what seems to be sclerotic tissue; whether pancreas tissue is contained is doubtful. Lymph glands of the mesentery of normal size, yellow in color on section; just below the stomach, at the base of the mesentery, is a necrotic lymph gland. Retro-peritoneal lymph glands possibly slightly enlarged, reddish on section. The lymphatics along the vena cava inferior are distended with clear yellow lymph. Spleen small, of normal color and consistency. Adrenals normal. Ureters slightly distended; kidneys show fatty degeneration of the cortex, capsule not adherent. Liver shows

REACTIONS OF THE BLOOD IN DIABETES MELLITUS. 269

extensive fatty metamorphosis. Heart and lungs normal. Aorta of a marked yellow color. Right eye contains a cataract, and the anterior chamber contains an hypopyon. Left eye appears normal, although the dog seemed to be blind for several days before death.

TABLE III.

Dog No. XIX. Large male mongrel. Pancreas removed April 2, 1903.

Date.	Amount in cc.	Fehling in %.	Ferment.	Total sugar.	Spec. grav.	Treatment.	Notes.
Apr. 3	550	2.8	15.4	1,028		
" 4	485	2.8	Posit.	16.6	1,022		
" 5	740	9.6	"	71.0	1,040		
" 6	450	8.0	"	34.0	1,046		
" 7	1,050	3.4	"	35.7	1,031	Wound dressed; has not united, but seems to be filled with clean granulation tissue.
" 8	550	9.6	"	52.8	1,046		
" 9	1,000	5.3	"	53.0	1,036	Bled.	
" 10	450	4.8	"	21.4	1,046	Dog eats 3 lbs. of meat per day.
" 11	1,100	5.3	"	58.3	1,042		
" 12	550	3.2	"	17.6	1,035	Urine contains quantities of fat floating on the surface; lipuria or from feces?
" 13	200	4.8	"	9.6	1,046		
" 14	285	9.6	"	25.8	1,046		
" 15	550	4.8	"	26.4	1,045		
" 16	1,000	3.2	"	32.0	1,046	Urine slightly tinged with red.
" 17	365	4.8	"	17.5	1,046	Bled.	Dog eats 4 lbs. of meat per day.
" 18	575	6.0	"	34.5	1,045		
" 19	270	5.6	"	15.1	1,046		
" 20	200	6.8	"	13.6	1,045	Dog becomes blind; diarrhea.
" 21	90	9.6	"	8.6	1,046	Dog eats very little, is very emaciated and very weak.
" 22	55	4.8	2.6			
" 23	No urine. Dog dies, blood collected immediately after death before coagulation takes place.						

Sections of the lymph glands and spleen of dog No. XIX. present a normal histologic picture. The liver and kidney present the same appearance as the same organs of dog No. VIII., except that the proportion of the degenerated liver cells

is greater than in dog No. VIII. In the liver of No. XIX. cells of normal appearance can sometimes be seen between degenerated cells. The fatty metamorphosis in this liver is more diffuse than in No. VIII., but here, too, the cells of the center of the acini show the most marked degeneration. The questionable tissue from the site of the pancreas proves to be granulation tissue; no pancreas cells are to be found.

III. 1.

THE HEMOLYTIC ACTION OF THE SERUM IN TRUE DIABETES MELLITUS.

The experiments upon the hemolytic action of the serum of the diabetic dogs were conducted each time under precisely the same conditions. A normal control dog and a diabetic dog were bled by exposing the jugular vein under cocaine, and inserting a large, sterile, hypodermic needle attached to a sterile flask or large test-tube, connected with an aspirating apparatus. At the same time a normal rabbit and a normal guinea-pig were bled, the bloods were defibrinated, and two and one-half cubic centimeters of each blood were placed in one hundred cubic centimeters of sterile 0.85 per cent sodium chloride solution in flasks graduated to fifty cubic centimeters. The following day the supernatant fluid was removed by means of a sterile syphon and the flasks filled with fresh sterile sodium chloride solution to the fifty cubic centimeters mark. The erythrocytes were in this manner washed in a great excess of salt solution. The following day four parallel series—two series of guinea-pig corpuscles, and two of rabbit corpuscles—of twelve or fifteen tubes in each series, were made, each tube containing one cubic centimeter of the five per cent suspensions of the erythrocytes. The series were compared after two hours at 37° C., and over night at low temperature.

The standard for comparing the hemolytic action of two or more sera may be either the point of beginning hemolysis, or the point of complete hemolysis, in the series made with the normal control serum. Either standard presents the difficulty that it is not always possible to determine just the point of beginning or the point of complete hemolysis. We have endeavored to obviate this difficulty, and to find an

accurate numerical expression of our results by recording the points of lowest hemolysis, and also the points of highest, respectively complete, hemolysis in the two series to be compared, reducing the result to a common standard, using 1 as the expression of the hemolysis of the normal series, and then drawing a mean between these two extremes. We have generally made the series with 0.01 to 0.12 cubic centimeters of the normal and of the diabetic serum.

Feb. 7, 1903:

Comparison of the hemolytic action of the serum of a normal dog with the serum of diabetic dog No. VIII., both dogs bled February 6. To two series of fifteen tubes, each tube containing 1.0 cc. of freshly washed guinea-pig's corpuscles, the normal, respectively diabetic serum, was added in the amounts 0.01 to 0.15 cc. To two parallel series of fifteen tubes, each tube containing 1.0 cc. of freshly washed rabbit's corpuscles, the normal, respectively diabetic, serum was added in the amounts 0.02 to 0.3 cc.

After two hours at 37° C. and over night in the ice-box, the following is recorded: Beginning hemolysis of 1.0 cc. of the 5 per cent suspension of guinea-pig's corpuscles is seen in the tube containing 0.01 cc. of the normal control serum; beginning hemolysis is seen in the tube containing 0.03 cc. of the diabetic serum. The same degree of nearly complete hemolysis of guinea-pig's corpuscles is produced by 0.1 cc. of the normal control serum and by 0.15 cc. of the diabetic serum.

Reducing these results:

Beginning hemolysis:	N.S.	0.01 cc.	=	0.03 cc. diabetic serum,
or,	N.S.	1	=	3 diabetic serum.
Highest hemolysis:	N.S.	0.1 cc.	=	0.15 cc. diabetic serum,
or,	N.S.	1	=	1.5 diabetic serum.

If we draw a mean between these two extremes of hemolysis, we find that N.S. 1 = 2.25 diabetic serum.

In other words, if 1 represents the amount of normal dog serum required to produce a certain amount of hemolysis of the corpuscles contained in 1.0 cc. of a 5 per cent suspension of washed guinea-pig's erythrocytes, then 2.25 times that amount of the diabetic serum will be required to produce the same degree of hemolysis of the same amount of corpuscles.

In the same way it was found that for rabbits' erythrocytes the relation was: N.S. 1 = 1.75 diabetic serum.

While such a method of expressing results is manifestly attended by certain sources of error, we believe that this method is the fairest possible for expressing comparative results. Since all the experiments were conducted under

precisely the same conditions we will omit a detailed description of them, and will present the results in the form of the following table:

Date of Bleeding.	Corpuscles.	Relative Hemolytic Action.			
Feb. 6th	G. P.	N.S.	$1 = 2.25$	serum No. 8	
	Rabbit	"	$1 = 1.75$	"	" 8
Feb. 11th	G. P.	"	$1 = 2.75$	"	" 8
	Rabbit	"	$1 = 2.1$	"	" 8
Feb. 19th	G. P.	"	$1 = 2.7$	"	" 8
	Rabbit	"	$1 = 2.45$	"	" 8
Feb. 21st	G. P.	"	$1 = 2.52$	"	" 8
	Rabbit	"	$1 = 2.83$	"	" 8
April 9th	G. P.	"	$1 = 4$	"	" 19
	Rabbit	"	$1 = 2$	"	" 19
April 17th	G. P.	"	$1 = 2.66$	"	" 19
	Rabbit	"	$1 = 2.6$	"	" 19

See Tables I. and II. for the daily records concerning these dogs. The same normal dog was used to furnish the control serum in three of the above experiments; two other normal dogs were used, one for each of two experiments, and for one experiment — February 11 — two series with the sera from two normal dogs were used as controls.

The loss of hemolytic property from the diabetic serum is, therefore, not to be explained as simulated by individual peculiarities of the sera of the control dogs, or by assuming individual differences in the susceptibility of the erythrocytes of the guinea-pigs and rabbits; a marked loss of hemolytic activity from the serum of two diabetic dogs was demonstrated, for the erythrocytes from six different normal guinea-pigs and six different normal rabbits, the standard of comparison being the sera obtained from five different normal dogs. This loss of hemolytic complements is, therefore, a constant phenomenon to be observed in the serum of a dog made diabetic by the complete removal of the pancreas.

Whether this loss of hemolytic activity is a progressive one or not is hard to answer with the data before us. From the table it will be seen that the loss of hemolytic power for rabbits' erythrocytes progressed from $1 = 1.75$ to $1 = 2.83$; but a similar progression in the loss of hemolytic activity for

guinea-pigs' corpuscles does not appear. It is possible that the use of different normal dogs as controls and of the erythrocytes from different animals may have obscured a progression in the recorded decrease of hemolytic power. On the whole, we incline to the opinion that this decrease does become more marked in the later stages of the disease.

It seemed possible to us that a progressive loss really existed, but was compensated by a progressive thickening of the diabetic serum. Determinations of the specific gravity of the normal and the diabetic serum by means of the pyknometer have shown that such a thickening of the serum of the diabetic does not occur; counts of the red blood cells also show no deviations from the normal, results which correspond with the recorded observations in human diabetes.

III. 2.

THE BACTERICIDAL ACTIVITY OF THE SERUM OF A DIABETIC DOG.

We have already stated our reasons for thinking that an analytic study of the phenomenon of bacteriolysis is impossible with our present knowledge. The experiments upon which we are about to report consist, therefore, of studies of the bactericidal effect of the serum of our diabetic dogs. The analogy which has been established between the phenomena of bacteriolysis and hemolysis by various workers under suitable conditions is sufficient warrant for the conclusion that the loss of bactericidal power from the serum of the diabetic dog which we have found is due to a loss of bacteriolytic complements.

We have compared the bactericidal power of the serum of the diabetic with that of the normal dog upon *B. coli communis*, *B. typhi abdominalis*, and *B. dysenteriae* (Shiga), and also upon two strains of *Staphylococcus pyogenes aureus* which had been isolated from dogs whose pancreas had been removed: *Staphylococcus* No. 1 was isolated from the peritoneum of dog No. XVIII., and was a strongly chromatogenous type; *Staphylococcus* No. 2 was isolated from the skin wound of dog No. XVII., and was marked by its slight

tendency to pigment production. As has been established by other workers, it is hardly possible to speak of a bactericidal effect of normal serum upon the staphylococci; we do see, however, that normal sera have a pronounced inhibitory action upon the growth of the organism, and this property of inhibiting the growth is more or less lost from the serum in diabetes.

The experiments were all conducted in exactly the same manner. Tubes containing the same quantities of 0.85 per cent sodium chloride solution, of the serum of the diabetic dog, and of the serum of the normal control dog, were inoculated with the same amounts of a young culture of the colon, typhoid, and dysentery bacilli, grown upon slant agar and suspended in 0.85 per cent sodium chloride solution; the staphylococcus was grown in flasks containing bouillon and sand, and the clumps were broken up by thorough shaking and filtering through a sterile asbestos glass wool filter. The inoculated tubes were left at 37° C. during the course of the experiment, and agar plates poured at the intervals noted in the tables.

Bactericidal action of diabetic serum, dog No. XIX.; bleeding of April 9, 1903.

	1 hour.	4 hours.	8 hours.	12 hours.	24 hours.
<i>Staphylococcus pyogenes aureus</i> , No. 1.					
NaCl	328	240	120	116	76
N.S.	448	394	9,000	(∞)	
No. 19	516	448	(∞)	(∞)	

<i>Staphylococcus pyogenes aureus</i> , No. 2.					
NaCl	488	500	400	440	292
N.S.	536	452	568	512	696
No. 19	576	660	684	1,698	(∞)

<i>B. typhi abdominalis</i> .					
NaCl	1,030	960	1,120	1,500	900
N.S.	438	69	55	10	1
No. 19	540	64	65	128	900

<i>B. dysenteriae</i> (Shiga).					
NaCl	450	240	510	620	270
N.S.	158	1	2	1	4
No. 19	759	12	1	1	1

This experiment illustrates our preliminary remarks in regard to the inhibitory effect of the normal dog's serum upon the growth of the *Staphylococcus*; this is more clearly seen in the case of the strain No. 2 than in No. 1. The serum of the diabetic dog No. XIX. has evidently lost a portion of its normal bactericidal action upon the bacillus of typhoid, while no effect is seen upon those substances in the serum which protect against dysentery. This result finds its explanation in the experiments performed in this laboratory by Vedder (loc. cit.), who found a marked difference in the behavior of the different bacteriolytic complements toward a porcelain filter; the complements for *Staphylococcus* and for colon were removed by passing the serum through a Berkefeld filter, while the complements for typhoid and sometimes for dysentery passed the pores of the filter, and were demonstrable in the filtrate.

Bactericidal effect of diabetic serum, dog No. XIX.; bleeding of April 17, 1903.

	1 hour.	8 hours.	12 hours.	24 hours.
<i>Staphylococcus pyogenes aureus</i> , No. 1.				
NaCl	88	129	64	1
N.S.	390	(∞)	(∞)	(∞)
No. 19	282	(∞)	(∞)	(∞)
<i>Staphylococcus pyogenes aureus</i> , No. 2.				
NaCl	400	160	0	0
N.S.	276	294	279	386
No. 19	430	660	470	1,990
<i>B. coli communis</i> .				
NaCl	1,460	1,800	2,100	2,956
N.S.	26	0	1	232
No. 19	1,320	20,000	6,200	10,000
<i>B. typhi abdominalis</i> .				
NaCl	1,220	1,910	1,740	2,290
N.S.	1,690	64	95	820
No. 19	1,790	(∞)	(∞)	(∞)
<i>B. dysenteriae</i> (Shiga).				
NaCl	11	1,750	1,150	1,340
N.S.	135	0	1?	0
No. 19	5	(∞)	(∞)	(∞)

In this experiment the difference between the effect of the normal serum upon the two strains of *Staphylococcus* is brought out very clearly. *Staphylococcus* No. 1 is evidently not in the least affected. To what this is due is a question beyond the province of our present work. We see further that the diabetic serum has now lost its protective property against the dysentery bacillus; the discrepancies of the plates of the first hour are evidently due to some error.

Bactericidal effect of diabetic serum, dog No. XIX.; blood collected April 23, 1903.

	1 hour.	7 hours.	24 hours.
<i>Staphylococcus pyogenes aureus</i> , No. 1.			
NaCl	430	360	Turbid.
N.S.	910	(∞)	"
No. 19	540	(∞)	"
<i>Staphylococcus pyogenes aureus</i> , No. 2.			
NaCl	210	220	20
N.S.	130	154	126
No. 19	138	1,640	(∞)
<i>B. coli communis</i> .			
NaCl	1,950	11,000	9,000
N.S.	75	0	0
No. 19	1,430	1,550	(∞)
<i>B. typhi abdominalis</i> .			
NaCl	4,080	4,020	4,200
N.S.	4,800	1,030	7,000
No. 19	3,390	(∞)	(∞)
<i>B. dysenteriae (Shiga)</i> .			
NaCl	550	290	270
N.S.	57	0	0
No. 19	750	(∞)	(∞)

The normal control dog used for this experiment was a different one from the animal whose serum furnished the control for the two previous experiments. With the exception of the *Staphylococcus*, we may conclude from these experiments that the diabetic serum has entirely lost its normal bactericidal property; from analogy with the hemolytic experiments we must also conclude that this loss is due to an entire absence from the serum of the diabetic dog of the normal bacteriolytic complements.

IV.

THE DECREASE IN THE NORMAL HEMOLYTIC ACTIVITY OF THE SERUM OF THE DIABETIC DOG IS DUE TO A LOSS OF COMPLEMENT.

It was first reported by Ehrlich and Morgenroth⁷ that fresh guinea-pig serum will reactivate heated normal dog's serum, provided that the guinea-pig serum be present in sufficient amounts. Having satisfied ourselves by a preliminary test that such is the case, we performed the following experiment:

April 23, 1903. — A number of normal guinea-pigs were bled and the serum allowed to separate; guinea-pig's blood was defibrinated, and both serum and blood placed in the ice-box. Blood was taken from the heart of dog No. XIX., and the serum collected. We would again call attention to the fact that, although the dog had died shortly before we were prepared to bleed it, the blood had not yet coagulated in the vessels. A normal dog, bled once two days before, was bled and the serum allowed to separate.

The following day the sera were tested as follows:

Series I.

G.P. serum. 1.0 cc.	G.P. blood. 1 drop.	Heated N.S. 0.05 cc.	0.85% NaCl. 0.35 cc.
"	"	0.1 "	0.3 "
"	"	0.15 "	0.25 "
"	"	0.2 "	0.2 "
"	"	0.25 "	0.15 "
"	"	0.3 "	0.1 "
"	"	0.4 "	

Series II. — A duplicate of Series I. in every respect, except that the heated serum of diabetic dog No. XIX. was substituted for the heated normal serum.

Controls: Two tubes each containing 1.0 cc. G.P. serum, plus 1 drop G.P. blood; two tubes each containing 0.5 cc. G.P. serum, plus 0.5 cc. of 0.85 per cent NaCl solution, plus 1 drop G.P. blood. Three tubes, each tube containing 1.0 cc. of 0.85 per cent NaCl solution, plus 1 drop G.P. blood, plus 0.1, 0.2, 0.4 cc. respectively of heated normal dog serum; three tubes containing exactly the same combination as the above three tubes, except that the heated diabetic serum was substituted for the heated normal serum.

Series III. — To each of twelve tubes, each tube containing 1.0 cc. of 0.85 per cent NaCl solution, was added one drop of defibrinated guinea-pig's blood; to these tubes were then added the following amounts of fresh normal dog serum: 0.01, — 0.02, — 0.03, — 0.04, — 0.05, — 0.06, — 0.07, — 0.08, — 0.09, — 0.1, — 0.11, — 0.12 cc.

Series IV. — A duplicate of Series III. in every respect, except that the fresh serum of diabetic dog No. XIX. was substituted for the normal fresh serum of Series III.

After two hours at 37° C. and over night at room temperature the following result is recorded :

Series I. — Reactivation of heated normal dog's serum ; hemolysis is clearly marked in the tube containing 0.05 cc. of the heated normal serum ; hemolysis is complete in the tube containing 0.3 cc. of the heated normal serum.

Series II. — Reactivation of heated serum of diabetic dog No. XIX. ; hemolysis is clearly marked in the tube containing 0.05 cc. of the heated diabetic serum ; hemolysis is complete in the tube containing 0.25 cc. of the heated diabetic serum.

In none of the control tubes is there the slightest evidence of hemolysis ; the heated normal serum and the heated diabetic serum show the same degree of agglutination.

Series III. — Fresh normal dog serum ; hemolysis is marked in the tube containing 0.01 cc. of the fresh normal serum, and increases rapidly to complete hemolysis in the tube containing 0.04 cc.

Series IV. — Fresh serum of diabetic dog No. XIX. ; no hemolysis in the tube containing 0.01 cc. of the diabetic serum ; hemolysis is complete in the tube containing 0.08 cc.

If it is assumed that the amboceptor which is reactivated by the fresh guinea-pig serum is identical with the amboceptor which acts upon the erythrocytes when the fresh serum is added, then it is not clear at first glance why the point of complete hemolysis should not be the same, or nearly the same, in both cases ; the fresh normal serum causes complete hemolysis of one drop of guinea-pig's blood in the amount of 0.04 cc., while 0.3 cc. of heated normal serum is required to cause complete hemolysis of the same amount of blood when reactivated by fresh guinea-pig serum. A similar difference exists between the action of the fresh and the reactivated heated diabetic serum. Kyes and Sachs⁸ emphasize the property of normal rabbit's serum which enables it to protect the erythrocytes of the same animal from the hemolytic action of cobra venom. It may be that the normal guinea-pig's serum exercises an analogous protective action upon the erythrocytes suspended in it, when these corpuscles are exposed to the action of the normal hemolytic amboceptors of dog's serum. We see further in this experiment the loss of hemolytic activity from the fresh diabetic serum, the relation being N.S. 1 = 2 diabetic serum.

The heated normal serum and the heated diabetic serum do not of themselves hemolyze guinea-pig's erythrocytes; but in the presence of fresh guinea-pig's serum these erythrocytes are hemolyzed by nearly the same amounts of heated normal and heated diabetic serum, the slight difference noted in the experiment in favor of the diabetic serum being probably referable to an individual difference in the amounts of hemolytic amboceptors contained in the sera from the two dogs. We must, therefore, conclude from these results that there is no change in the serum content of hemolytic amboceptors in diabetes, and, consequently, that the loss of hemolytic activity is due to a decrease of hemolytic complements. By analogy with the hemolytic process the loss of bactericidal power is doubtless due to the decrease of bacteriolytic complements.

We have endeavored to solve this problem by attempting to separate the hemolytic amboceptor from its complement by means of the dialyzer; we have dialyzed normal dog's serum against constantly changing isotonic and hypotonic salt solutions, but have not obtained the slightest evidence of a loss of amboceptors from the dialyzed serum. We have further attempted to separate the two factors by saturation with erythrocytes in the cold, but with no success. An apparatus for maintaining a constant and low temperature was not at our disposal; but dog's serum is so actively hemolytic for the erythrocytes used in our work that very active hemolysis occurs even when the erythrocytes are added to cooled serum and the whole is placed in thin-walled test-tubes in direct contact with the ice of an ice-chest. We have also attempted to remove the amboceptors by the following method: A small test-tube was placed in the aluminum casing of the centrifuge, and the space between the tube and the casing was filled with water; the casing was then placed in freezing mixture until it was surrounded with a layer of firm ice. Dog's serum was then placed in the tube and erythrocytes from the rabbit or guinea-pig added in the proportion of one cubic centimeter erythrocytes to two cubic centimeters of serum. The ice casing is not melted in the

revolving centrifuge until after the corpuscles have been thrown to the bottom of the tube. As soon as the corpuscles, aided by the agglutination, were separated, the supernatant fluid was removed and placed in a fresh ice-cased tube, fresh corpuscles were added, and the operation was repeated. Everything was of course kept sterile. This was repeated as many as four times, agglutination being marked each time. A certain dilution of the dog's serum must necessarily occur because of the addition of the salt solution contained between the corpuscles which were added. A very slight reduction of hemolytic activity was obtained, but so slight as to be more readily explained by this dilution than by assuming a removal of the amboceptors by the saturation.

V.

IS THE COMPLETE REMOVAL OF THE PANCREAS NECESSARY TO THE LOSS OF COMPLEMENTS IN EXPERIMENTAL DIABETES?

TABLE IV.

Dog No. XVI. Very small mongrel bitch. Pancreas removed March 16, 1903.

Date.	Amount in cc.	Fehl. in per cent.	Ferment.	Total sugar.	Spec. grav.	Treat. ment.	Notes.
Mar. 17	200	16.6	Pos.	33.2	1.048		
" 18	240	16.2	38.9	1.047		
" 19	245	15.6	38.7	1.048		
" 20	205	16.1	33.0	1.048		
" 21	325	5.6	18.2	1.038		
" 22							
" 23	335	6.09	20.4	1.046	Dog eats very little; drinks a little water.
" 24	100	6.8	6.8	1.048	
" 25	90	5.0	4.5	Bled.	
" 26	40	1.0	0.4			
" 27	28	1.0	0.3	Left eye closed by purulent discharge.
" 28	80	1.6	1.3	Bled and killed.	

Autopsy. Body of a very small emaciated bitch. There is a small suppurating wound of the skin of the neck, at the site of bleeding. Abdominal skin wound open, and suppurating along the deeper sutures. Peritoneum normal. A portion of pancreas tissue is found adherent to the linea alba, in the fat of the peritoneal fold which is normally attached to the linea alba of dogs. No pancreas to be found at the normal site of that organ. Spleen, adrenals, and lymph glands are normal; some of the lymph glands seem slightly soft and congested, but not enough to be called abnormal. Kidneys show fatty degeneration of the cortex; capsule not adherent. Liver of normal size, slightly yellow. Lungs and heart normal. The fat of the entire body, including the subcutaneous fat of the limbs, shows great numbers of small, whitish foci.

Sections of the spleen of dog No. XVI. show normal histologic structure. Kidney shows no striking changes. The liver shows some cells in the center of the acini which have undergone fatty metamorphosis; these cells occur side by side with normal cells.

TABLE V.

Dog No. XX. Large male mongrel. Pancreas removed April 7, 1903.

Date.	Amount in cc.	Fehl. in per cent.	Ferment.	Spec. grav.	Treatment.	Notes.
April 8	No urine.					
" 9	400	Neg.	Neg.	1,025	The amount of urine voided was never abnormal; color and odor normal.
" 10	400	"	"	1,015		
" 12	"	"	1,032		
" 13	"	1,029		
" 14	"	1,027	Placed in an ordinary cage and urine not further observed until marked emaciation.
May 1	"	Neg.	1,031		
" 4	"	"	1,036	Polarimeter shows slight laevorotation. Dog very emaciated; stools white with fats.
" 5	"	1,036	Bled and killed.	

Autopsy. Dog is very emaciated and weak, and is killed. The superficial layers of the skin of the back are suppurating in several places; the affection is strictly local and does not extend into the subcutaneous tissue. The wound at the site of operation has healed perfectly. In the right groin is a blister-like collection of yellowish fluid, and there is a similar, smaller lesion in the left groin. The wound of the linea alba has healed perfectly. Peritoneum normal; very little fat in the mesentery; mesenteric lymph glands not enlarged. Retroperitoneal lymph glands possibly slightly larger than normal, otherwise normal. Spleen normal in size, color, and on section. Capsule of the kidneys not adherent,

cortex rather pale. Adrenals normal. No pancreas tissue to be found at the normal site of the organ; attached to the greater curvature of the stomach, and extending into the omentum, is a small oval piece of tissue, about one and one-half cm. long, one cm. wide, and not more than one mm. in thickness; frozen sections of this suspected tissue show it to be pancreas tissue. The liver is large, of uniform yellow color, probably due in greater part to the anemia produced by bleeding the dog to death. Heart and lungs are normal.

Microscopic study of sections of the organs of dog No. XX. shows that the organs are entirely normal, except for a congestion of the liver capillaries.

While we have performed no direct experiments upon this phase of our problem, the frequent occurrence of aberrant islands of pancreatic tissue has made it possible to study this question in two of our dogs. The first instance of the incomplete removal of the pancreas which we encountered was in dog No. XVI. As will be seen from the protocol, a very marked excretion of glucose immediately followed the operation, amounting to sixteen and six-tenths per cent for the first twenty-four hours, equivalent to a total excretion of thirty-three and two-tenths grams of sugar. In the succeeding eleven days the amount of excreted glucose gradually fell to about one per cent per day, and the dog was killed. At autopsy a small bit of pancreas tissue was found adherent to the linea alba; this tissue gave the impression of having been detached from the main portion of the organ at the operation, and of having been left free in the peritoneal cavity to later attach itself to the place in which it was found. We cannot say whether this is a possible explanation or not. The dog was first bled after the disease had existed for nine days; the hemolytic test of the serum showed a relation of N.S. 1 = 2.5 diabetic serum No. XVI. for guinea-pigs' erythrocytes, and of N.S. 1 = 3 diabetic serum for rabbits' corpuscles. Three days later the sugar excretion had fallen from five per cent to one and six-tenths per cent, and the animal was bled and killed. The hemolytic test now showed a relation of N.S. 1 = 2.2 diabetic serum for guinea-pigs' corpuscles, and of N.S. 1 = 1.8 diabetic serum for rabbits' erythrocytes.

The bactericidal test of the serum of dog No. XVI., obtained at the bleeding of March twenty-fifth, showed the

following results. The figures in the table represent a mean between the counts of two duplicate plates made from each tube at the several periods :

	1 hour.	8 hours.	24 hours.	48 hours.
<i>Staphylococcus pyogenes aureus</i> (stock culture).				
NaCl	266	0	0	
N.S.	191	1,800	(∞)	
No. 16	416	738	(∞)	
<i>B. typhi abdominalis</i> .				
NaCl	1,224	912	1,109	1,240
N.S.	944	86	167	5,000
No. 16	894	28	178	(∞)
<i>B. coli communis</i> .				
NaCl	598	2,040	7,560	6,500
N.S.	292	1.5	62.5	(∞)
No. 16	310	32	844	(∞)
<i>B. dysenteriae</i> (Shiga).				
NaCl	684	843	584	664
N.S.	520	0.5	0	0.5
No. 16	1,004	1	2.5	0

The diabetes in this dog had progressed for only nine days ; the serum of the diabetic dog No. XIX. showed very little loss of bactericidal power after a diabetes of seven days, but after fifteen days the diabetic serum of No. XIX. showed a marked loss of bactericidal activity. Possibly the diabetes in this dog, No. XVI., had not existed long enough to cause a loss of bactericidal properties. A bactericidal test of the serum obtained from this dog at the time the animal was killed was, unfortunately, not made ; we present this table, therefore, merely as an addition to our knowledge of the behavior of the bactericidal properties of the serum in diabetes.

While the results obtained with the serum of dog No. XVI. are not at all conclusive, the experiments with another dog, No. XX., seem to us to point to but one conclusion, namely, that the complete removal of the pancreas is as necessary to cause a loss of the complements as it is to the production of a true diabetes. The pancreas of dog No. XX. was removed

on April 7, 1903. The urine was examined during the following six days, but no trace of sugar was found. The dog was then placed in another cage. At the beginning of May it was noticed that the stools of this dog contained great amounts of fat, and that the dog had become greatly emaciated. The urine was again examined, but still no trace of sugar could be found; examination with the polarimeter gave a slight degree of laevorotation. May 5, twenty-eight days after the operation, the dog had become so emaciated and weak that it could stand only with difficulty, and was bled and killed. The hemolytic test showed a relation of N.S. 1 = 1.68 diabetic serum No. XX. for guinea-pig's erythrocytes; no difference could be seen between the hemolytic activity of the normal control serum and that of the diabetic serum for rabbit's erythrocytes. A bactericidal test of this serum upon *B. coli communis* and *B. typhi abdominalis* gave the following results:

	1 hour.	6 hours.	24 hours.
<i>B. coli communis.</i>			
NaCl	2,370	2,200	20,000
N.S.	24	3	2
No. 20	21	3	0
<i>B. typhi abdominalis.</i>			
NaCl	3,000	1,460	1,500
N.S.	2,140	147	99
No. 20	3,250	56	210

At autopsy a very small bit of pancreas tissue was found attached to the greater curvature of the stomach and extending for a short distance into the omentum. The animal had become as emaciated as the dogs with typical diabetes, proving that nutrition had been seriously impaired; the metabolism of the fats at least had been as defective as in typical diabetes, judging from the appearance of the stools and from the fat floating upon the surface of the urine. In other words, the normal functions of the pancreas, except that of preventing a diabetes, were lost; and yet it is evidently impossible to speak of a loss of complements, either hemolytic or bacteriolytic, from the serum of this dog, for the slight loss of

hemolytic activity for guinea-pig's erythrocytes is not greater than might be seen between two normal dogs. Another evidence that the bacteriolytic complements had not materially decreased is the fact that the wound had healed perfectly, and had not subsequently opened. The evidence offered by the experiments with both dogs No. XVI. and No. XX. seems to justify the conclusion that the complete extirpation of the pancreas is necessary to the marked decrease of the complements which characterizes diabetes mellitus.

VI.

HAS THE COMPLETE REMOVAL OF THE PANCREAS DEPRIVED THE ORGANISM OF ITS POWER TO REACT TO AN INFLAMMATORY PROCESS BY AN INCREASE OF THE COMPLEMENTS ?

TABLE VI.

Dog No. XIII. Small mongrel bitch. Pancreas removed Feb. 26, 1903. Weight before operation, 13 lbs. Weight after death, 9 lbs.

Date.	Amount in cc.	Fehl. in per cent.	Ferment.	Total sugar.	Spec. grav.	Treat- ment.	Notes.
Feb. 27	100	10.6	Pos.	10.6			
" 28	335	10.7	"	35.8	1,050		
Mar. 1	300	4.8	"	14.4	1,033		
" 2	60	8.33	"	5.0			
" 3	115	10.0	"	11.5	1,050		
" 4	730	3.13	"	22.8	1,045	Bled.	
" 5	600	4.17	"	25.0	1,032		
" 6	375	8.33	"	31.2	1,050		
" 7	470	12.8	"	60.1	1,050	Bled.	
" 8	260	10.0	"	26.0	1,050	Stools are typical of diabetes, containing great amounts of fat.
" 9	440	5.0	"	22.0	1,048		
" 10	140	4.0	"	5.6	1,050		
" 11	Dead.						

Autopsy. Small, very much emaciated bitch. The external wound shows some suppuration; several of the deeper sutures form the focus of slight suppurative processes. Peritoneum normal. No suppuration at the site of the pancreas, and no pancreas tissue to be found. Lymph glands not enlarged, of normal color and consistency; spleen normal, also adrenals. Liver adherent to site of pancreas, is of normal color, and shows no fatty degeneration on macroscopic examination. Kidneys pale, capsule not adherent. Heart and lungs normal. The diaphragmatic and mediastinal pleuræ are covered with the fibrinous exudate of a pleuritis; the remainder of the pleura appears normal. Sections of the organs of dog No. XIII. show no marked histologic changes. Kidney shows some parenchymatous degeneration. The liver cells seem narrower than normal, but do not exhibit any fatty metamorphosis.

The results obtained with the serum of but one dog have a direct bearing upon this question. The course of the disease in this animal, dog No. XIII., had been perfectly typical until two days before death, when the amount of excreted glucose fell. The autopsy revealed a purulent pleuritis as the immediate cause of death; no trace of pancreas tissue could be found. We are aware of the fact that the result noted in one animal is not as conclusive as the results obtained from a series of animals would be; but we nevertheless consider the result in this one case of sufficient importance to explain its publication, and when we consider the result in the light of our entire knowledge of the question of the complements, it seems as though a definite conclusion were well warranted.

The serum obtained at the first bleeding of diabetic dog No. XIII. on March fourth, 1903, showed a loss of hemolytic activity for both rabbit's and guinea-pig's erythrocytes which can be expressed in accordance with the methods outlined above by a relation of N.S. $1 = 1.75$ diabetic serum No. XIII. The diabetic dog No. XIII. was bled again three days later, and the hemolytic test showed no difference between the degree of hemolysis caused by the normal control serum and that caused by the diabetic serum. The normal dog, which furnished the control serum for this second test, was not the same animal as the one used for the test of March fourth, since we had not anticipated the result; but it was the same dog which was used to furnish the control serum for a number of the experiments with normal and diabetic

sera, and a deviation in the hemolytic action of this normal serum was never observed. We therefore believe that the diabetic serum must have regained its normal content of hemolytic substances after the loss shown in the first test. Bactericidal experiments were made with the sera obtained at both these bleedings, but absolutely no loss of bactericidal activity was observed.

Four days after the second tests were made the dog (No. XIII.) died, and the autopsy revealed a purulent pleuritis. It is a well-established fact that there occurs, coincident with the inflammatory process, an increase of complementary substances. We therefore conclude that the primary loss of hemolytic complement in this animal was compensated by the increase of complement occurring hand in hand with the inflammatory process of the pleuritis. We are not surprised at the fact that no loss of bactericidal activity was seen, since we have shown that the bacteriolytic complements are not lost as early in the disease as are the hemolytic complements. About the time that a decrease of bactericidal activity might have been expected, had the disease run an uncomplicated course, the disease process caused an increase of complements, and the result of our second bactericidal experiment was also negative.

The results of this experiment offer at least a fruitful suggestion for further work. In our own mind we are satisfied that the results show that the complete removal of the pancreas has not deprived the organism of its power to react normally to the inflammatory process.

VII.

CAN AN ABNORMAL AVIDITY OF THE RECEPTOR GROUPS OF THE DIABETIC ERYTHROCYTES BE DEMONSTRATED?

According to the theories embodied in the teachings of Ehrlich, another possibility must be considered in the question of the loss of natural resistance in diabetes mellitus. Such an increase of susceptibility may be due to defects in the amboceptor or a reduction of complement, or to the

acquisition by the body cells of an abnormal degree of receptivity for the invading parasites or their products. The last of these possibilities seems to find a possible basis in the abnormal staining reaction of the diabetic erythrocytes, first described by Bremer.⁹ While later work has shown that the reaction is not specific, it does seem to indicate an abnormal condition of the erythrocytes in diabetes. We do not mean to draw the conclusion that a deviation from the normal reaction of a group of cells towards a stain would necessarily mean that the receptors of those cells had suffered an alteration; but the fact of Bremer's reaction points to some change in the constitution of the red blood cells, and suggests the possibility that the biologic reaction of the cell to a specific toxin might evidence more clearly and more specifically the change which has taken place. The diagnostic value of an eventual positive result is self-evident.

It seemed theoretically possible to detect an abnormality of the receptor avidity by subjecting both normal and diabetic erythrocytes to the action of the same toxic agent and comparing the results. In our work we have made 5 per cent suspensions of normal and of diabetic erythrocytes, and have then made hemolytic series with the serum of a rabbit highly immunized against dog's blood, as follows:

Feb. 6, 1903. — Five per cent suspensions in 0.85 per cent NaCl solution were made of the washed erythrocytes of a normal dog and of the diabetic dog No. VIII. The specific hemolytic serum used for determining the relative resistance of the normal and diabetic cells was furnished by a rabbit which had been immunized by intra-peritoneal injections of a total of 79.5 cc. of defibrinated dog's blood. The manner of making the test was to add to two series of fifteen tubes of each of the blood suspensions, each tube containing 1.0 cc. of the suspension, the same amounts of the specific hemolytic serum, increasing regularly through the fifteen tubes from 0.01 to 0.15 cc.

After the customary procedure of two hours at 37° C. and over night in the ice-chest, there is no appreciable difference in the degree of hemolysis exhibited by the two series. Hemolysis is very marked in the tubes of both the normal and the diabetic series to which 0.01 cc. of the specific serum was added; complete hemolysis occurs in both series in the tubes containing 0.1 cc. of the specific serum.

It is true that in this experiment the diabetes had not been of long standing. The experiment was repeated in exactly the same manner with the erythrocytes of this same dog, obtained immediately before death from typical diabetes of eighteen days' duration. The specific immune serum for this experiment was obtained from a rabbit which had received intra-peritoneally a total of 104.5 cc. of defibrinated dog's blood. Hemolysis in both normal and the diabetic series was again identical. In both these experiments the normal erythrocytes and the diabetic erythrocytes were agglutinated in the same degree.

The result of these experiments is, therefore, identical with that reported by Kyes and Sachs (*loc. cit.*) upon the susceptibility of the erythrocytes from the human diabetic to the action of cobra venom. They report that the erythrocytes from several healthy subjects, from two cases of diabetes, one pneumonia and one typhoid, have shown no difference in susceptibility to the hemolytic action of cobra venom.

VIII.

THE RELATION OF THE LEUCOCYTE TO THE LOSS OF COMPLEMENTS IN DIABETES.

We have shown that a marked loss of hemolytic and, doubtless, also of bacteriolytic complements occurs in the advanced stages of experimental diabetes mellitus. Although we have previously published the results of the work⁴ upon which our conclusion was based, — that no form of the leucocyte is concerned in the elaboration of the hemolytic complements, — and although our work with the hemolytic complements has been borne out in every detail by the work of Vedder upon the bacteriolytic complements,² and by the work of others,¹⁰ we have considered that we have in diabetes mellitus an unstudied and a peculiarly adapted field for the consideration of the assumed relation of the leucocyte to the production of the complements.

If it were held that the leucocytes excrete complement during life, this phase of our problem would be more difficult

of solution; but the generally accepted doctrine, that of Metschnikoff and the French school, is that the complements are first set free after the destruction of the leucocyte, which takes place with the coagulation of the blood. One of the following three propositions must, therefore, be true for diabetes mellitus: Either the diabetic blood contains fewer leucocytes than normal; or the leucocytes of the diabetic organism are, in some unknown way, deprived of this one of their functions — the production of complements; or else we must assume that the leucocytes are in no way concerned in the formation of these substances.

1. Does the blood of the diabetic contain fewer leucocytes than normal? The following blood counts bear upon this point. The counts were made from the blood of the diabetic dog No. XIX., and compared with the counts made from the blood of the same normal dog, both counts being made under the same conditions of time, and so forth.

April 6, 1903.	11 A.M.	Normal dog.	15,600.
		Dog No. XIX.	16,840.
April 7.	10 A.M.	Normal dog.	13,400.
		Dog No. XIX.	20,360.
April 8.	10 A.M.	Normal dog.	16,300.
		Dog No. XIX.	17,950.

April 9. At time of bleeding. These counts were made from the blood of the jugular veins.

		Normal dog.	9,400.
		Dog No. XIX.	17,600.
April 10.	3.30 P.M.	Normal dog.	12,400.
		Dog No. XIX.	22,750.
April 15.		Dog No. XIX.	28,050.

April 17. At time of bleeding.

Peripheral blood of normal dog.	15,100.
Blood from jugular vein of dog No. XIX.	17,150.

The serum obtained at the bleeding of April ninth gave the following hemolytic results: For the guinea-pig's corpuscles the serum of the normal dog was four times as active as the serum of the diabetic dog No. XIX; for rabbit's corpuscles the normal serum was twice as active as the diabetic serum. Bactericidal experiments showed practically

no difference in the effect of the two sera. At the bleeding of April seventeenth the normal serum was found to be nearly three times as actively hemolytic for both guinea-pig's and for rabbit's corpuscles as the serum of the diabetic dog No. XIX. Bactericidal tests (see p. 275) now gave a result which can only be interpreted as due to an entire loss of bacteriolytic complements from the diabetic serum.

If the leucocyte is the source of the complements, the diabetic serum should have been more active, both as regards hemolysis and as regards bacteriolysis, for it contained a greater number of leucocytes. A differential count showed practically no deviation of the diabetic blood from the normal in the matter of the percentage relation of mononuclears to polynuclears.

These results are supported entirely by the clinical observations upon human diabetes. We have found no records in the special text-books upon the pathology of the blood, nor in the works of our best authorities upon diabetes mellitus of any clinical observations of either a hyperleucocytosis or of a hypoleucocytosis in the course of diabetes. That the diabetic dog in our experiments showed a greater or less degree of hyperleucocytosis is not to be wondered at, since inflammatory processes unavoidably accompany the healing of the wound and are continued by the suppuration which marks the slight tendency to wound healing. None of our sections of the lymph glands have shown evidences of either hypertrophic or of atrophic processes; no mitotic figures are seen; the spleen is of normal size, color, consistency, and microscopic structure. If the leucocytes were the source of the complements we should naturally expect some evidence of an attempt on the part of the leucocytic complex to counteract the loss of complement, and to repair the loss by a compensatory hypertrophy; but no evidence of such an attempt can be found. Clinical observations and our own work have, therefore, shown that the leucocytes are not reduced in numbers in the diabetic organism, yet we find a loss of over sixty per cent of the hemolytic complements, and, apparently, an entire loss of the bacteriolytic complements.

2. Have we evidence of any interference with the normal functions of the leucocytes in diabetes? Differential stains of blood smears have shown no deviations from the normal, except in one particular; eosinophile polynuclears are quite common in normal dog's blood, while we have not found a single eosinophile in our preparations of diabetic blood. The percentage relation of mononuclears to polynuclears in the diabetic blood is practically the same as in the normal blood. This result is evidently borne out by clinical observations, for we find no record of any characteristic or constant change in the leucocytes of human diabetic blood.

In a review of our work,⁴ upon which was based the conclusion that the leucocytes are not concerned in the production of the hemolytic complements, M. Besredka¹¹ explains our results as follows: "Dans les conditions où s'est placé S., il est certain que la grande majorité des mononucléaires de son exsudat étaient en plein travail de phagocytose; occupés à digérer les leucocytes polynucléaires, ces mononucléaires peuvent être considérés comme n'étant plus bons à faire autre chose; il n'y a pas donc lieu de s'étonner qu'un pareil exsudat ne se montre pas doué du pouvoir hémolysant (en présence du fixateur ou de l'ambocepteur); il n'est pas dit qu'un exsudat mononucléaire frais, normal, ne puisse renfermer le complément hémolytique tout comme un ganglion lymphatique de cobaye renfermer un complément vis-à-vis des globules rouges de lapin, par exemple."

We grant the point emphasized by Besredka, that the "macrocytes" were engaged in phagocytosis of the "microcytes"; but we know of no physiologic parallel for the statement that an organ loses one normal function simply because it is engaged in performing another equally normal function. Further, the fundamental fact that complement production is a normal function of the leucocytes still remains unproven. In diabetes, however, there can evidently be no question of phagocytosis, for the simple reason that there is nothing to be engulfed. The possibility exists that the polynuclears could be engaged in phagocytosis of

the fat granules which sometimes appear in diabetic blood in the form of a pronounced lipemia. But in the case of diabetic dog No. VIII. no lipemia was observed at any of the four bleedings; the serum was perfectly clear and of a deep golden-yellow color, very like the serum of the duck. Yet this dog showed as marked a loss of complements as did dog No. XIX., the blood of which exhibited a marked lipemia.

It has been found, however, that the leucocytes of the diabetic organism contain glycogen. This was demonstrated long ago by Gabritschewski¹² and also by Minkowski.⁶ But if we assume that the taking up of glycogen paralyzes the normal complement-producing function of the leucocytes, we immediately prove that the leucocytes in inflammatory processes cannot produce complements. It is demonstrated in an admirable study by Best¹³ that glycogen, respectively a glycogen compound, is a constant finding in the leucocytes in inflammatory conditions. "Die jodempfindliche Substanz . . . ist ferner Bestandtheil der polynucleären Leucocyten, aber auch der Gewebe und Gewebszellen bei acuten, weniger bei chronischen Entzündungen und Eiterungen. Sie entsteht als Reaction auf positiv chemotaktisch wirkende Stoffe und Bakteriengifte. Sie ist sicher als solche kein Degenerations-zeichen, eher ein Zeichen erhöhter Activität der Zellen" (loc. cit., p. 603). Others consider the presence of glycogen in the leucocyte to be a sign of degeneration; ^{14, 15} a discussion of this question would be out of place here, but we would state that no one has advanced any reason for assuming that the presence of glycogen in the leucocytes is the effect of different processes in diabetes and in inflammation.

If, then, the presence of glycogen in the leucocyte can prevent the production of complement in diabetes, the same must be true in ordinary acute inflammation; yet the theory of Metschnikoff is that the increase of complements coincident with an inflammation is due to an increase of leucocytic activity. Consequently the adherents of this teaching cannot grant that the glycogen content of the leucocytes in inflammation prevents a production of complements, and therefore

we must conclude that the glycogen could not prevent the formation of the complements by the leucocytes of the diabetic organism.

The removal of the pancreas has of itself, so far as has ever been recorded by those who have worked in this field, no effect upon the leucocytic complex. That the general metabolic activity of the entire organism, and probably also of the leucocytes, is impaired, is true; but in the case of our dog No. XX. the same effect as seen in diabetes upon the general metabolism of the body was noted, evidenced by the faulty digestion of fats, progressive emaciation, and muscular weakness. No excretion of glucose was recorded, also no loss of complements. We must therefore conclude that impaired metabolism cannot of itself cause the loss of complements — unless we wish to assume that the metabolism of sugar and complement production are intimately associated. The conclusion that the leucocytes are in no way concerned in the production of the complements seems inevitable.

IX.

THE EFFECT OF AN INTERCURRENT INFECTION UPON THE EXCRETION OF GLUCOSE.

It is a commonly accepted teaching of clinical medicine, apparently well founded (see ¹⁶), that the amount of glucose excreted by the diabetic organism is decreased by an intercurrent infectious process. This phenomenon is generally thought to have some connection with the concomitant fever of the infectious process, and has been variously explained. It does not seem worth while to enter into a discussion of the theories which have been advanced so long as direct experiment and the collateral evidence obtained in the study of other phases of the experimental diabetes in animals does not uphold the fact of a decreased excretion during an infection. The only direct experiments which we have encountered in the literature are those reported by Nebelthau,¹⁷ who has studied the effect upon the excretion of glucose in dogs made diabetic by the complete removal of the pancreas, of injections of diphtheria toxin, tetanus toxin, diphtheria

cultures in bouillon, living tubercle bacilli, and living streptococci, and in dogs in which an accidental peritonitis followed the operation. Nebelthau concludes that the fever subsequent to the introduction of bacterial toxins into the organism, as well as the fever of acute infectious processes, does not of itself necessarily influence the carbohydrate metabolism of the diabetic dog. Under the influence of tuberculosis, on the other hand, the diabetic organism may find time and opportunity to develop powers which may make possible a decrease of the amount of glucose excreted.

While we have conducted no direct experiments upon the relation between infection and the excretion of glucose in the diabetic dog, such an experimental problem as ours has presented the opportunity for the study of this question, since the animals are so extremely susceptible to infection. Six of our dogs lived long enough to assure us of the fact that the entire pancreas had been removed and that a true diabetes, varying slightly in severity, had begun; they then succumbed after a longer or shorter period to the effects of infectious processes, in some cases of a more general character, in other cases more or less localized. The following are the records of the dogs in question, with which that of diabetic dog No. XIII. (see p. 285) may be included:

TABLE VII.
Dog No. XI. Pancreas removed February 13.

Date.	Amount in cc.	Fehl. in per cent.	Ferment.	Total sugar.	Spec. grav.	Notes.
Feb. 14	No urine.					
" 15	550	4.36	Pos.	24.0		
" 16	425	1.5	"	6.4		
" 17	425	1.34	"	5.7		
" 18	350	1.4	"	4.9		
" 19	Dead. At autopsy an abscess enclosed by omentum and intestines is found at the site of the pancreas. Wound of skin suppurating. Organs show no striking abnormalities. No trace of pancreas tissue.					

Dog No. XII. Pancreas removed February 24.

Feb. 25	350	12.0	Pos.	42.0		
" 26	700	8.0	"	56.0	1,039	
" 27	1,330	4.8	"	63.8	1,040	
" 28	520	3.2	"	16.6	1,030	
Mar. 1	150	8.0	"	12.0	1,050	Urine cherry red, — hemoglobin?
" 2	60	16.0	Outlet of cage stopped.		
" 3	265	4.17	11.0	Dead. Phlegmonous abscess of the subcutaneous tissue of the left side, extending through the muscles to the peritoneum. Intestinal peritoneum slightly roughened, not congested. Suppuration of the wound in several places, extending through both layers of sutures; localized abscess of surface of the liver; small foci of suppuration at the site of the pancreas. Liver and kidneys show fatty degeneration. Other organs normal. No trace of pancreas tissue.

Dog No. XIV. Pancreas removed February 27.

Feb. 28	450	7.4	Pos.	33.3	1,050	
Mar. 1	600	2.4	"	14.4	1,023	
" 2	600	2.5	"	15.0	1,029	Beaker overflows, urine lost; amount lost ?
" 3	750	1.43	"	10.7	1,020	
" 4	Dead. Slight suppuration of the wound of skin, with several foci of suppuration of the deeper layer of sutures. No peritonitis; foci of suppuration at the site of the pancreas. Liver and kidneys show slight degree of fatty degeneration; other organs normal. No trace of pancreas tissue.					

Dog No. XV. Pancreas removed March 12.

Mar. 13	350	7.14	Pos.	25.0	1,039	
" 14	385	16.7	"	64.3	1,050	
" 15	375	12.0	"	45.0	1,042	
" 16	250	8.3	"	20.7	1,043	
" 17	Dead. Purulent peritonitis. Liver of yellowish color. Other organs apparently normal, except for the congestion due to the peritonitis. No trace of pancreas tissue.					

Dog No. XVII. Pancreas removed March 30.

Mar. 31	185	8.7	Pos.	16.1	1,045	
April 1	560	9.6	"	53.8	1,048	
" 2	200	8.7	"	17.4	1,046	
" 3	5.6	Outlet of cage stopped, only few cc. examined.			
" 4	1,200	1.2	Pos.	14.4	1,010	
" 5	335	4.8	"	15.3	1,036	Feces contain blood.
" 6	Dead. Autopsy shows general peritonitis. Intestines filled with blood-stained matter. Prolapsus recti. A variety of <i>Staphylococcus pyogenes aureus</i> , marked by slight tendency to pigment production, grows upon cultures made from the peritoneum. Organs appear normal except for the immediate changes due to the peritonitis. No trace of pancreas tissue.					

A study of these protocols shows that a decrease of sugar production does actually occur, although it will be seen from the records of the two dogs in which the diabetes ran an uncomplicated course that the enormous output of the first few days tends to decrease. This is to be expected, since the first effect of the removal of the pancreas is to cause the excretion of the sugar stored in the organism; later the excreted glucose must come from the disturbed metabolism of the carbohydrates. It must further be remembered that there invariably occurs with the onset of the acute stage of the infection a marked loss of appetite, the dogs as a rule eating nothing whatever. It is, therefore, not surprising that a decrease in the metabolic processes occurs. We are inclined to the belief that the infection in our own cases had of itself no direct influence upon the decrease of sugar excretion, and that the conclusion reached by Nebelthau is the correct one. The indirect influence through the inhibition of metabolic processes cannot be denied. Our experiments extended over only the earlier stages of the disease, and we are not prepared to say that an infection occurring in the late stages of experimental diabetes would not affect the excretion of glucose.

X.

CONCLUSIONS.

The difficulties encountered in the course of such an investigation have necessarily made it impossible for us to obtain results from a large series of animals; but we believe that the work above reported in detail fully justifies the following conclusions:

1. The subcutaneous injection of an alcoholic solution of phlorizin, which causes a transitory glycosuria, is followed by a slight, though readily demonstrable, increase in the serum of the rabbit of the hemolytic complement for bovine erythrocytes; this increase is to be explained as occurring coincidently with the inflammatory reaction of the organism to the injection.

2. No effect of injections of phlorizin upon the amboceptor for bovine erythrocytes can be demonstrated.

3. The intraperitoneal injection of adrenalin chloride is followed by no marked effect upon the blood reactions; the injection may, however, cause an inflammatory reaction, and so cause an increase of complementary activity.

4. The complete removal of the pancreas from dogs, which causes a true diabetes mellitus of severe type, is followed by a marked decrease of the hemolytic activity of the diabetic dog's serum for both rabbits' and guinea-pigs' erythrocytes.

5. The diabetes caused by the complete extirpation of the pancreas is further characterized by what is to be interpreted as a complete loss of the normal bactericidal property of the serum of the dog; this can be demonstrated conclusively for *B. coli communis*, *B. typhi abdominalis*, and for *B. dysenteriae* (Shiga). Less conclusive is the demonstration of a decrease of bactericidal power of the diabetic serum for *Staphylococcus pyogenes aureus*, for the reason that the normal serum of the dog has very little, if any, bactericidal effect upon this organism.

6. This decrease of hemolytic activity of the serum of the diabetic dog is due to loss of hemolytic complements.

The loss of bactericidal power is, from analogy with the hemolytic phenomenon, doubtless to be interpreted as due to a loss of bacteriolytic complements.

7. The complete removal of the pancreas is as necessary to this loss of complements as it is to the production of a diabetes.

8. The complete removal of the pancreas has not deprived the organism of its power to react to the inflammatory process by an increase of the complementary substances.

9. No disturbance of the normal relation of the receptors of the erythrocytes to specific hemolytic amboceptors can be demonstrated in the course of a true experimental diabetes.

10. The loss of the complementary substances in diabetes mellitus points conclusively to the fact that no relation exists between the leucocytes of any type and the production of the complements.

11. A decrease in the amount of glucose excreted by the diabetic organism cannot be shown to occur in the course of a secondary infection, at least during the earlier stages of the diabetes.

(It is with pleasure that I express my indebtedness to Dr. A. C. Abbott, Director of the Laboratory of Hygiene of the University of Pennsylvania, for the suggestion which led to the work, and for extending to me the privileges of the laboratory. I am further indebted to Dr. Abbott for the interest which he has manifested, and for his personal assistance at all times.

I would also acknowledge my thanks to my friend, Mr. J. H. Fager, of the Class of 1904, Medical, for his assistance in the greater number of the tedious operations.)

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THE PATHOLOGY OF CHRONIC FLUORINE POISONING.¹

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The literature of fluorine poisoning is not very rich. Rabuteau, and later Tappeiner and Hewelke, worked out the toxicology of sodium fluoride, which is used for preserving food and beverages. Tappeiner found that 0.15 gram for one thousand grams of an animal's weight, subcutaneously injected, is to be considered a toxic dose, and leads to salivation, increased action of the lachrymal glands, drowsiness, muscular twitching, and spasms. (Toxic dose per os 0.5 gram per kilogram.) On giving small doses for a protracted period, Tappeiner and Brandl found an accumulation of fluorine in the bones in the form of a crystalline compound, which they considered as calcium fluoride. Acute fluorine poisoning in man has been described several times. Professor Nichles lost his life by inhaling the vapor of hydro-fluoric acid. Cameron saw two workmen in a lime factory killed by inhaling silicium fluoride, or more probably hydro-fluoric acid.

The writer of this article was the first to present a case of chronic fluorine poisoning in man brought about by taking up the poison in food.

For details of this case I must refer the reader to the New York Medical Journal of July 6, 1901.

Summary.—A man thirty-three years of age, a heavy smoker, and for years a beer drinker, taking from six to ten bottles of beer a day, from the same brewer and bottler, developed multiple phlebitis, without apparent etiology, and later severe pains in his bones. The ordinary clinical examination gave no result, but finally I discovered the clotting power of the blood to be greatly increased—one-half to one minute, instead of about five minutes. From the works of Wright, Pastellano, Limbeck, Green, Ringer, and Sainsbury, I knew that an increase of lime salts in the blood was capable of increasing its clotting power, and found, indeed, the dry residue of the blood as 14 per cent, instead of 1 per cent of the weight of the fresh blood. Urine and feces showed an excess of lime, the former containing almost no chloride. The relative blood count exhibited a great

¹ Received for publication June 24, 1903.

increase of uninuclear leucocytes and a specially great number of myelocytes.

During the analysis for lime salts the presence of fluorine in the blood and urine could be demonstrated. In looking for the source of this poison, I found that the beer contained a small quantity of it, and thereby could prove that my patient for a long time had been taking a small amount of fluoride of sodium daily. He developed an illness the chief symptoms of which were uninuclear leucocytes, lack of chlorine in the system, increased excretion of calcium in the feces and urine, increased coagulability of the blood, pains in the bones as in osteomalacea.

The opinions concerning fluorine are very diverse among educated people. I received a number of inquiries from chemists and pharmacists asking whether fluorine is toxic. The American Brewers' Journal published a caustic retort, in which they protested that the fluorine could not be traced in the beer. Yet in the same number this paper advertised all possible preservatives of beer, and among them recommends sodium fluoride as absolutely safe.

The toxicology of fluorine is entirely untried. Hydrofluoric is known as a highly dangerous gas, and its solution, fluoric acid, causes severe burning on the skin, and, frequently, ulcers on the cornea of those who work with it. (Tappeiner, *Archives for Experimental Pathology*, 25, 203 (1889); and 27, 108 (1890); Scholz, *idem*, 25, 326 (1889).) Inhalations of the fumes of hydrofluoric acid and other fluorine combinations were recommended for diphtheria and tuberculosis, but were soon declared useless. (Granches, Poriac, Chautard, Hoerman, Opolsky.)

It still remained for me to convince even the most skeptical, by experiments on animals, that fluorine produced in them the same symptoms as in man.

My method for determining the clotting time of the blood is described in my former paper.

Chronic fluorine poisoning in animals. — I very slowly and gradually treated a number of rabbits, guinea-pigs, and pigeons with sodium fluoride.

RABBITS. — Each of them received daily three centigrams of sodium fluoride mixed with their food. After a week the animals began to be careful in the use of their legs; they inclined to running eyes, and several got diarrhea.

From time to time I made blood examinations, especially for leucocytes and for coagulability. Finally I killed one after the other of the animals, in order to make a thorough analysis of their blood and a careful examination of their bones.

Let us consider first the relative number of leucocytes and the clotting power of the blood. The nomenclature of hematology is not absolutely well-defined, and I therefore have to make a few remarks regarding it.

Among leucocytes we distinguish uninuclear and multinuclear cells. The uninuclear group comprises the small lymphocytes, about seven mikrons in diameter; the medium ones of about twice that size; the large ones about twenty to thirty mikrons, and the myelocytes or Ranvier's *celles médulaires*, from ten to thirty mikrons. The latter have large nuclei that can be stained only with difficulty, while the nuclei of the lymphocytes have a great affinity to basic colors like methylene blue.

The protoplasm of the myelocytes is frequently more basophile than the nuclei; often it contains neutrophile and eosinophile granulations. The protoplasm of the lymphocytes is generally smooth. The multinuclear group comprises the polynuclear, the polymorphous, and the transitory forms, but these three kinds of cells are not distinctly separated from each other and vary in sound animals. The lymphocytes, too, seem to be entirely individual in their relative numbers. The myelocytes are absent in the normal blood of rabbits, guinea-pigs, and also of man.

To save time I will give the main results of the experiments on rabbits in a table.

BLOOD OF FLUORINE RABBIT.

Leucocytes.	Normal Rabbit.	I.	II.	III.	IV.	V.	VI.
Uninuclear	40-45%	57%	70%	73.5%	60%	89%	90%
Small	16	22	4	5.5	22	Almost all myelocytes.	All myelocytes.
Medium	19	9	7	22	3		
Large	8	2	2	8	—		
Myelocytes	—	24	57	38	35		
Multinuclear	60-55	43	30	26.5	40	11%	10%
Polynuclear	32-35	17	27	24.5	35	11	10
Polymorphous and transitory forms	24-26	26	3	2	5		
Coagulability of total blood in open vessel	6-8 minutes.	4 minutes.	—	2½ minutes.	3 minutes.	3½ minutes.	2½ minutes.
Blood in capillary	5 minutes.	50 seconds.	1½ minutes.	¾ minute.	Poikilocytosis. Myelocytes very small, severe diarrhea.	Arthritis. Megalocytes, nucleated erythrocytes.	But faint trace of chloride in urine.
Remarks	No trace of chloride in urine.			

These rabbits had received for a period of from ten to twenty days a daily dose of 0.03 gram sodium fluoride each. During the first days no symptoms were observable, but gradually the animals developed considerable anemia; the eyes of most of them watered very easily; some of them lost their appetite and became very emaciated. As before mentioned, they seemed to avoid unnecessary movements.

After killing the animals for my final examinations I opened the still beating heart so as to procure as much blood as possible through the contractions. I thus had an opportunity of observing the coagulation of a large quantity of blood, and found the time almost always shorter than in normal animals.

In several instances I collected the total amount of blood in three more or less equal parts, and noticed that the fluorine blood still possessed the same quality as the normal; namely, that the last third clots in about one-quarter or one-third of the time that is necessary for the first part.

In the above table it is shown that in every instance the percentage of uninuclear leucocytes had greatly increased, the average number of uninuclear forms being seventy-three per cent. Still more remarkable than this increase is the outnumbering of the myelocytes, a form of leucocytes very rare in normal rabbits. In my fluorine rabbits the average percentage of these is a little more than fifty per cent of all the leucocytes. I consider this point the most characteristic symptom of chronic fluorine poisoning.

Several of the animals showed marked anemia, one of them a pronounced polyarthritis, while each fluorine animal shows redness in one or more of the joints. The clotting power of the blood appears to be increased, though in many of the animals it varies little from the normal. In two guinea-pigs the clotting was one-half and three-fourths of the normal, while the percentages of leucocytes and of myelocytes were similar to those found in rabbits.

The urine in most of the animals showed a lack or absence of chlorine.

Rabbit bones. — Macroscopically the periosteum of the bones of fluorine rabbits showed no changes. The marrow of the tubular bones showed a color similar to raspberry jelly. Fresh microscopical preparations exhibit lymphocytes and myelocytes and a few fatty cells. In one case I found crystalline formations in the shape of disks, columns, etc., resembling the fluor-calcium crystals produced in the test-tubes.

Decalcified bones, preserved in formalin, show in microscopical sections marrow with numerous cells. Between the cells there is a granular mass. These grains are insoluble in acetic and hydrochloric acids and consist, perhaps, of precipitated calcium fluoride. Such granulations, however, can always be found in cases of severe disease of the bone marrow, that is to say, when the latter is in a state of fatty degeneration and granulating necrosis. The capillaries are filled to the utmost, even in animals that bled to death. Scattered through the texture, but mainly attached to the spongy spicules, are giant cells now and then in excavations of the bone lamellæ, as osteoclasts. There is no sign of regeneration of the bones. The periosteum is more cellular than normal.

The bones of several of the animals, sound and poisoned, were preserved, thoroughly cleaned, and then their specific gravity ascertained with this result:

Poisoned.	Normal.
sp. gr. 1.011	1.265 air-dried, unopened.
“ “ 1.176	1.286 “ opened.

From this will be seen that the bones of the fluorine-animals were considerably lighter than those of the sound rabbits.

In the internal organs of the rabbits treated with fluoride I have found nothing characteristic; stomach and intestines show slight catarrhal changes.

PIGEONS. — A number of pigeons received, in their food, granules made of paste containing a small percentage of

sodium fluoride — about one-fifth of a milligram each. The pigeons, therefore, had an opportunity to eat about two milligrams a day, but they certainly did not eat all the granules offered to them daily. After thirty to sixty days they were examined and then killed. Compared with healthy pigeons they seemed rather sickly. They were unnaturally quiet, the feathers were slightly ruffled, the feet and bills roughened, and the eyes red. One pigeon even had a slight ulcer on the cornea. The blood drawn from a toe showed increased clotting power. In one of the pigeons it clotted solidly in less than two minutes. Microscopical preparations of the blood showed the erythrocytes unchanged in form, but their nuclei seemed to be a shade less blue than in the blood of normal pigeons. (This is mentioned only as a casual observation, and is not to be considered as a standard symptom of fluorine poisoning.) There were very few leucocytes and almost all of them uninuclear. The greater part of the uninuclear forms were to be called large lymphocytes with oval and but faintly bluish nuclei, the protoplasm being colorless and not granulated. The smaller percentage of the uninuclear forms were regular myelocytes, with a round and very pale blue nucleus surrounded by a small body which stained pale purple in Jenner's solution. Transitory forms and multinuclear cells were very few — about five or ten per cent of all the leucocytes.

The livers of the pigeons showed slight fatty degeneration; the stomach and intestines marked catarrhal changes, but the main attack of the fluoride of sodium seems to have been directed against the osseous system. The periosteum of the tubular bones was decidedly redder and more sanguineous than the periosteum of normal bones. The bone marrow of the femur and tibia was bright red, with almost a purplish tint (raspberry color), while the normal marrow had a more yellowish and less reddish shade. Microscopically the transversal section showed in the fluorine pigeons an almost total absence of fat tissue, while normal pigeons have a considerable amount of it. The capillaries were filled to their utmost capacity, the tissue itself consisted chiefly of

round cells, partly small, with a moderately well-stained nucleus, partly of the type of myelocytes or lymphocytes; besides that, there was a mass of fine granules which filled every free space. Nowhere could I find crystalline products; in the sections of decalcified bones there seemed to be a large increase of osteoclasts, as if a very active resorption of bone were going on. The breast bone of the pigeon which looked sickest was in parts totally decalcified, resembling a membrane more than a bone. This cannot have been an accident, because all the pigeons were of the same age and not young enough to have such soft bones. The femur and tibia of normal and fluorine pigeons were carefully scraped, and then the specific gravity determined.

Normal.		Fluorine.	
Femur, air-dried,	1.338	Femur, air-dried,	1.319
Tibia "	1.172	Tibia "	1.125

(Remark.) In spite of the fact, therefore, that the change of the marrow from yellow into red eliminates the light fat cells, the bone of the fluorine animal had lost quite an amount in its specific gravity.

The conclusions to be drawn from the experiments with animals are the following:

After ingesting fluoride for several weeks the animals show variations in the percentage of the leucocytes in favor of the uninuclear forms. The further the poisoning advances, the more will the uninuclear cells adopt the form of myelocytes, and finally none but myelocytes can be found, the nuclei of which can hardly be stained. Aside from them there are, in extreme cases, only a few — barely ten per cent — neutrophile polynuclear cells. The latter, too, exhibit a lack of chromatin substance. The source of the myelocytes is beyond doubt the bone marrow, which is in a condition of considerable irritation, or proliferating inflammation. It is evident that there is considerable resorption in the bones in progress. This latter assumption is corroborated by the

difference of the specific gravity of the sound and the poisoned bones. Furthermore, the blood of the fluorine rabbits and pigeons shows a very high degree of coagulability. From four-fifths to one-fifth only of the normal time is required for coagulation. The increase of coagulability, apparently, does not depend on the proportion of the leucocytes. While the animal is bleeding to death, the coagulability of the blood of fluorine animals increases as rapidly as in the blood of normal rabbits towards the latter portions of the blood. The last third requires hardly one-half the time to coagulate that the first half does. The leucocytes remain during the bleeding in approximately equal proportions.

I have not observed thrombosis in any of the animals, and I scarcely expected to find it. A rabbit of one or two years of age offers in this respect totally different conditions from those prevalent in a man of thirty-three, aside from the difference in the length of the veins.

I cannot report regarding the excretion of calcium by the rabbits and pigeons. Investigations of this kind require very much time, and on account of the minute quantities — say about two to five milligrams per day — they are very inexact. Besides, I have as yet been unable to find a record of the normal quantities of calcium or chloride excretion of rabbits and pigeons.

My experiments with animals have thus far yielded in all the principal questions exactly the same results as were obtained by my observations of the fluorine patient. I therefore consider the pathology of the chronic fluorine poisoning as determined: The sodium fluoride passes, as such, into the blood and with this into the bone marrow. There the fluorine finds a substance with which it enters into a very firm and almost insoluble combination, namely, the calcium of the bone, and, in accordance with well-known chemical laws, severs its weaker combination with the sodium. Calcium is present in the bone probably as a complex substance, as a double salt of calcium with phosphoric acid and chlorine similar to the mineral apatite. The latter

is very likely replaced by the fluorine in certain molecular proportions, and in this way a substance is formed which, when found in nature, is called fluorapatit. Then the chlorine, freed from its calcium combination, combines with the sodium, freed from the fluorine, and is lost through the kidneys. In all probability this entire process is much more complex in reality than I am here able to suggest, and this subject requires profound study and investigation before it will become clear.

The increase of calcium in the blood and the resultant greater calcium excretion I consider a consequence of the proliferation of lymphoid tissue in the marrow and an effect of the fluorine poisoning. At the same time, a small quantity of calcium may enter the blood as calcium fluoride, in which it is soluble in the proportion of about one to thirty thousand.

The excretion of chloride and calcium may be placed in a new light by an article of Heidenhein (Pflueger's Archive, 56, pages 579, 894). This investigator discovered that sodium fluoride arrests the chemical action of the epithelium of the intestines, and that their resorptive activity is very much delayed thereby. Aside from this, the affected layer of epithelium allows salts from the blood to transude into the contents of the intestine, a process never tolerated by normal, healthy intestinal epithelium. In this way chlorine and also calcium may be lost. (Quoted from W. Heidenhein, Significance of the Jonen Theory, 1902.)

Chronic fluorine poisoning is of special interest, because it creates microscopic pictures in the blood which can be found elsewhere only in leukemia. The difference becomes evident, of course, when one counts the leucocytes. In fluorine poisoning their number is not much larger than normal.

In summing up, I wish to state that the main symptoms of chronic fluorine poisoning in man and animals so far experimented upon are the same: Abnormal increase of the myelocytes in the blood at expense of the number of the other leucocytes, partial change of the yellow bone marrow into red (so far seen only in animals, as my patient did not die),

pain in the bones and joints, increased coagulability of the blood, and great deficiency of chlorine in the body.

That there was an enormous increase in the output of calcium could be shown directly in man through the analysis of the urine and feces, and in animals by determining the specific gravity of the bones.

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CONTENTS.

	PAGE
OBSERVATIONS ON THE MORPHOLOGY OF BACILLUS TUBERCULOSIS FROM HUMAN AND BOVINE SOURCES. (With ten plates.) 4-13	
<i>S. B. Wolbach and H. C. Ernst</i>	313
A HITHERTO UNDESCRIBED FIBRILLAR SUBSTANCE PRODUCED BY CONNECTIVE-TISSUE CELLS. (With one plate.) 14	
<i>F. B. Mallory</i>	334
ON THE APPEARANCE AND SIGNIFICANCE OF CERTAIN GRANULES IN THE ERYTHROCYTES OF MAN. (With two plates in colors.) 15-16	
<i>V. C. Vaughan, Jr.</i>	342
A STUDY OF THE VOLUME INDEX. OBSERVATIONS UPON THE VOLUME OF ERYTHROCYTES IN VARIOUS DISEASE CONDITIONS.	
<i>J. A. Capps</i>	367
BACTERIUM PYOGENES SANGUINARIUM. (With one plate.) 17	
<i>N. L. Berry, Jr., and H. C. Ernst</i>	402
THE INFLUENCE OF CERTAIN BACTERIA ON THE COAGULATION OF THE BLOOD.	
<i>Leo Loeb</i>	407
OBSERVATIONS, ESPECIALLY WITH THE ROENTGEN RAYS, ON THE ARTIFICIALLY DEFORMED FOOT OF THE CHINESE LADY OF RANK, IN RELATION TO THE FUNCTIONAL PATHOGENESIS OF DEFORMITY. (With nine plates.) 18-26	
<i>P. E. Brown</i>	420
METHODS OF TREATMENT OF CONGENITAL DISLOCATION OF THE HIP. <i>E. H. Bradford</i>	433
RESISTANCE OF THE MUSCLES IN REDUCING A CONGENITALLY DISLOCATED HIP.	
<i>E. H. Bradford and L. T. Wilson</i>	437
MECHANISM FOR REDUCING CONGENITALLY DISLOCATED HIP. (With two plates.) 26-26 ^b	
<i>Ralph W. Bartlett</i>	440
ON THE OUTPUT OF AMMONIA IN THE COURSE OF DIFFERENT FORMS OF INSANITY.	
<i>P. A. Levene and L. B. Stookey</i>	449
THE PASSAGE OF TUBERCLE BACILLI THROUGH THE NORMAL INTESTINAL WALL. (A preliminary report.)	
<i>M. P. Ravenel</i>	460
THE OCCURRENCE OF THE COLON BACILLUS ON THE HANDS.	
<i>C. E. A. Winslow</i>	463
PROTOZOA IN A CASE OF TROPICAL ULCER ("DELHI SORE"). (With four plates.)	
<i>J. H. Wright</i> 27-30	472

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No. 3.

OBSERVATIONS ON THE MORPHOLOGY OF BACILLUS TUBERCULOSIS FROM HUMAN AND BOVINE SOURCES.*

S. B. WOLBACH, M.D.,† AND HAROLD C. ERNST, M. D.

(From the Bacteriological Laboratory of the Harvard Medical School.)

That the tubercle bacillus as found in the discharges of tuberculous subjects and in pure cultures presents a great variety of appearances is a matter of common observation. Even in a single cover-glass preparation from a pure culture there may be marked variation in size and staining reaction.

When grown on different media and under different conditions as regards temperature and supply of oxygen this range of variation is indeed most extensive. Thus we may have cultures yielding only exceedingly short rods and micrococcus forms less than one micron in length, or cultures yielding almost wholly branch and thread-like forms ten to thirty microns in length.

The results obtained by different workers with pure cultures and with experimental lesions in rabbits have quite definitely proven the relationship of the tubercle bacillus with the higher bacteria. It was not our purpose in beginning this work to obtain additional evidence of this relationship. Our object was to make a study of the variations occurring in pure cultures at different ages and on different media, and at the same time to make a comparison of the human and bovine bacilli when grown under these different conditions. Incidentally, the results have furnished confirmatory evidence of the relationship of the tubercle bacillus, both human and bovine, with the higher bacteria.

Eleven pure cultures were obtained — four of human origin, seven of bovine. With the exception of one bovine culture

* Received for publication Sept. 4, 1903.

† Rockefeller Research scholar.

from Kral, they were all isolated by one of us during the last two years.

HUMAN CULTURES.

1. Tuberculous testicle. Culture obtained directly on human blood serum January 2, 1902.
2. Tuberculous testicle. Culture obtained directly on Dorset's egg medium March 26, 1903.
3. Urine from case diagnosed as tuberculous kidney. Culture on Dorset's egg medium from inoculated guinea-pig November 23, 1902.
4. Sputum from advanced case of pulmonary tuberculosis. Culture obtained on Dorset's egg medium from inoculated guinea-pig February 17, 1903.

BOVINE CULTURES.

1. Bovine culture from Kral's laboratory. Received on glycerine agar, but as transfers were unsuccessful, a guinea-pig was inoculated and cultures obtained from it on Dorset's egg medium.
2. Cow killed by the Massachusetts Cattle Commission. Disease was well advanced. Culture obtained from a nodule in one lung directly on Loeffler's blood serum January 29, 1903.
- 3.* Cow killed by the Massachusetts Cattle Commission. Beginning pulmonary tuberculosis. Culture obtained directly on Dorset's egg medium January 1, 1903.
- 4.* Cow killed by Massachusetts Cattle Commission. Beginning pulmonary tuberculosis. Culture obtained directly on Dorset's egg medium January 10, 1903.
5. Cow killed at the Brighton Abattoir. Advanced tuberculosis. Culture obtained on Dorset's egg medium from inoculated guinea-pig May 5, 1902.
- 6.* Cow killed by Massachusetts Cattle Commission. Beginning pulmonary tuberculosis. Culture obtained directly on Dorset's egg medium January 21, 1903.
- 7.* Cow killed by Massachusetts Cattle Commission.

* These four cows were killed November 18, 1902, having reacted to tuberculin a few days previous. One year before did not react to tuberculin.

Beginning pulmonary tuberculosis. Culture obtained from inoculated guinea-pig on Dorset's egg medium January 21, 1903.

VIRULENCE.

Bovine culture 2 and Kral and human culture 1 (testicle 1) were of proved virulence to guinea-pigs and rabbits. Bovine cultures 4, 5, and 7 and human cultures 3 and 4 were proved strongly virulent for guinea-pigs.

STUDY OF CULTURES AT DIFFERENT AGES.

All of the eleven cultures were studied in this part of our work.

Technic. — The technic employed was substantially that of Dr. F. P. Denney in the study of the bacillus diphtheriæ.¹

Sterile bouillon, instead of water, was used to make the emulsions for inoculating the tubes.

The medium used was Dorset's whole egg medium,²⁶ hardened and sterilized at 70–80° C. The reaction was purposely left unchanged and ranged from one-fourth per cent alkaline to two per cent acid to phenolphthalein according to the age of the eggs.

The cultures used to inoculate the tubes were purposely taken from different media, glycerine agar, brain agar, blood serum (human and bovine) and egg at different ages, from a few weeks up to a few months.

Emulsions of the growth were made in sterile bouillon and egg tubes inoculated by rubbing three to five loopfuls, according to the size of the tube, over the surface of the medium.

The preparations with a few exceptions were made at intervals of two days for the first few weeks and then at longer intervals, five to ten days, for a few weeks longer. Different parts of the surface of the egg were used for each preparation, avoiding the dried upper part and the lower moist part above the water of condensation.

Cover-glass preparations were made in water on cover-glasses, the masses of bacilli being well broken up with a flattened platinum wire, and dried at room temperature.

The cover-glasses were then fixed in a twenty per cent solution of formalin in sixty per cent alcohol for thirty seconds, washed and stained for twenty minutes in cold carbol-fuchsin, decolorized for ten seconds in Orth's fluid, washed and allowed to dry spontaneously. In a number of series cover-glasses were also made, using the Ziehl-Nielsen stain after fixation in the flame. This was done for comparison, and though no important differences were observed, the first method was adhered to because of inviting less criticism.

Neither the age of the culture used nor the medium on which it was grown affected the growth or microscopic appearances of the sub-cultures on egg. Likewise the reaction of the egg medium had no effect on the general characteristics and changes occurring at different ages.

All tubes were sealed either with paraffin or with rubber caps and incubated at 37–38° C.

Measurements of the individual bacilli were in most instances computed from negatives — photomicrographs at one thousand diameters. In a few instances a Bausch and Lomb filar micrometer ocular was used. When both methods were used the results closely agreed.

The changes about to be described occurred with all the cultures used, though requiring different lengths of time for their completion — varying from fourteen to twenty-six days.

CULTURES OF BOVINE ORIGIN.

(Plates IV. and V.) First week. — Growth at the end of the first week usually just becomes apparent as a diffuse dulling of the surface of the medium. Cover-glass preparations on the fourth day, before any growth is visible, show that multiplication has actively begun, the preparations showing clumps of bacilli, each clump probably the descendants of a single bacillus. At this stage the rods are most often irregularly stained (barred or beaded), and relatively quite long — two or three microns. (Fig. 49, Plate XII.)

At the end of the first week branched bacilli were found in three of the seven cultures, and, as the photomicrographs

show, these branched bacilli are much longer than those filling most of the field, which have an average length of between one and a half and two microns, and are very often in pairs, arranged end to end. These pairs usually lie parallel and grouped in masses. The staining reaction varies greatly, as in all very young cultures, about half of the bacilli take the stain deeply, while the other half stain faintly. The method of staining has no effect on this difference of reaction.

Second week. — During the second week there is a marked tendency towards shorter forms, and the arrangement in pairs and parallel groups is lost. Most of the rods are faintly stained and contain a deeply stained, spherical or oval body which may be at one end or in the middle of the rod. Occasionally two such bodies are found in the same faintly-stained rod. Generally the faintly-stained rods have the appearance of being mere appendages to the deeply-stained bodies, being ill defined, tapering off and ending in points.

The size of these bodies varies from a sphere whose diameter equals that of the rod or slightly greater to an oval bacillus-like body one micron long, occupying nearly the whole of the faintly-stained rod. These bodies, then, were it not for the faintly-stained tapering end, would be indistinguishable from the characteristic bovine bacillus on Dorset's egg medium at a later stage of growth. More suggestive still is the frequent occurrence of two such oval bodies end to end, one of which has attached to it a faintly-stained tapering rod, as if division had occurred. Less often are found chains of these bodies several microns long, all in the same faintly-stained rod. These deeply-stained bodies, which very closely resemble spores, are found in greater abundance in cultures, human and bovine, grown on brain and will be discussed under the description of these cultures.

Third week. — At this stage we begin to find mostly solidly stained, short, comparatively thick bacilli; in brief, the characteristic growth of fully developed cultures on egg. The length is strikingly shorter, and is found during the first ten days of growth, now averaging but slightly over one micron

in length. There is a great abundance of coccus and diplococcus forms.

Branched bacilli and the streptococcus-like chains were never found after the second week of growth, save in one culture, bovine 5, where such forms persisted.

CULTURES OF HUMAN ORIGIN.

(Plate VI.) First week. — Growth becomes visible in from five to eight days, as a diffuse dulling of the surface of the medium. Cover-glasses made on the fourth day show the bacilli in small clumps, similar to the bovine cultures at this stage. The rods are arranged end to end in parallel clumps. Lengths run from two to four microns. The rods are mostly solidly stained, a few granular ones being present in each field. Occasional chains of three bacilli occur. On the sixth or eighth day growth is visible, and the bacilli are found in large rope-like masses, representing small colonies, similar to the appearances obtained in impression preparations. Many faintly-stained rods are present.

Second week. — Shorter forms are now apparent, between one and two microns being usual. Occasional very short, deeply-stained and longer faintly-stained rods are found, the latter often containing one or two deeply-stained spherical bodies. Though faintly-stained rods are almost as numerous as in the early bovine cultures, the presence of the deeply-stained bodies in them is much less frequent. The end to end arrangement and the rope-like masses are still present.

Third week. — At the end of the third week we find the characteristic forms of a fully grown colony — short, comparatively thick, deeply-stained bacilli, averaging about one micron in length, and indistinguishable from the bovine cultures of the same age.

Up to about the third or fourth week the gross appearances of bovine and human cultures on egg are almost identical. At this time, however, the human cultures increase more rapidly and lose their moist appearance, which the bovine cultures retain.

Another fairly constant difference is in the density of the

two cultures. The fully grown human culture on egg is invariably hard and difficult to remove from the surface of the medium. In water, on making cover-glass preparations, it is difficult to break up, and it separates into smaller, equally resistant masses. The bovine cultures are much softer and mold easier with the platinum wire, also breaking up more easily when making cover-glass preparations.

The irregularly-stained rods described as occurring during the first few days of growth, and the barred bacilli and the coccus-like chains have also been described by A. von Huellen.² He speaks of colonies from a single bacillus entirely made up of such forms when examined at the third or fifth day of growth on Hesse's nutrient medium. This, he says, is the expression of unusually vigorous growth, and that these forms are not to be considered as "Dauerformen;" views with which we are in perfect accord.

To summarize, we may state that during the first days of growth the form assumed by the tubercle bacillus is longer than that of later stages, and that these forms grow progressively shorter until the type form of the fully developed culture is reached.

The irregularly-stained and branched forms occur at a period of vigorous multiplication, and cannot, therefore, be considered as involution or degenerate forms.

CHANGES OCCURRING AS A RESULT OF GROWTH ON DIFFERENT MEDIA.

In the present study only vigorously growing cultures, grown at 37-38° C., on media not altered by chemicals, were examined.

The cultures most carefully examined were a human culture (human 1) and a bovine culture (bovine 1), these being the first cultures isolated by us in the present work. All of the cultures, however, were grown on many of the media and all on both brain and egg, these media yielding the extremes of variation.

The following media were used: 1, glycerine agar; 2, beef serum hardened and sterilized at 100° C., with and without the addition of two per cent glycerine; 3, Loeffler's blood serum; 4, glycerine veal bouillon; 5, Dorset's egg medium; 6, human brain.

The human brain medium is that described by Ficker,³ excepting that we found it advisable before sterilizing to cover the slabs of brain with a five per cent solution of glycerine in water. Before inoculating the fluid is poured off.

As Theobald Smith⁴ has observed, the tubercle bacillus, like most bacteria, varies slightly in size and staining reaction from generation to generation. Therefore the following descriptions are given as a result of the examinations of many preparations, from many generations on each medium used.

It is not our intention to enter into a minute description of the cultures on each medium, but only to demonstrate the fact that very important changes do occur with changes of medium, and that this fact should be taken into consideration in describing cultures.

DORSET'S EGG MEDIUM.

(Figs. 18, 19, 20-21.) The differences in gross appearances of all human and bovine cultures grown on this medium were constant and striking. Eleven lots of eggs were used, varying in reaction from three-tenths per cent alkaline to two and two-tenths per cent acid to phenolphthalein, and the differences to be described were exhibited on all.

The human cultures yield more profuse growths. In both human and bovine cultures the growth spreads rapidly as a layer over the surface of the medium. The human cultures heap up more into irregular, knobbed masses which tend earlier to take a brownish pink coloration than in the case of the bovine cultures. The layer formed by human cultures is always dry looking and white, while that of the bovine culture is moist and translucent unless of considerable depth. In cultures one to two months old the heaped up masses of the bovine cultures are of a purer white than in the human cultures, which always have some color.

The microscopic appearance of both varieties grown on egg have been sufficiently described in the first part of this paper. Briefly, in fully developed cultures the microscopic characteristics are almost identical. In both, the short, relatively thick, deeply-stained bacilli predominate, the length averaging about one micron, the thickness one-third to one-half the length. Many perfectly round coccus forms are seen, and often these occur in pairs simulating diplococci.

In the human cultures there is always a slightly greater variation in length and thickness than in the bovine cultures. One bovine culture (5) persistently showed great variation in size, shape, and staining reaction. Though most of the bacilli were the usual short thick sort, long and even branched bacilli were found, as well as streptococcus-like chains of three to eight spherical deeply-stained bodies of varying sizes.

GLYCERINE AGAR.

(Figs. 22 and 23.) Seven per cent glycerine agar, made with meat extract and with veal broth, was used. The reaction at different times was adjusted to one and two-tenths per cent, one and three-tenths per cent, and one and five-tenths per cent acid to phenolphthalein. Thus far only three human and three bovine cultures have been made to grow on glycerine agar.

The bovine cultures, with the exception of the culture from Kral, grew less rapidly than the human cultures. All the bovine cultures preserved a more moist appearance and remained a purer white than the human cultures, with the exception of the culture from Kral, which acquired a delicate brownish pink tint.

The human cultures spread over the surface of the medium faster than did the bovine and became wrinkled and convoluted to a greater degree. Cultures one month old generally have acquired a brownish pink color on the older parts.

The microscopic appearances of cultures on glycerine agar are too well known to warrant a detailed description here. The differences observed between the human and bovine cultures are: 1. An average shorter length for the bovine

bacilli. 2. Greater variation in staining reaction, with an abundance of the deeply-stained oval and round bodies in fainter-stained bacilli before described.

In a twelve days' old bovine culture (4), at which time the growth had just become visible as a translucent grayish layer, unusually long bacilli were found, three to six microns. These bacilli stained but faintly, contained the deeply-stained bodies in all shapes, and, as will be described later in the description of cultures grown on the brain medium, many of these bodies were eccentrically placed. (Fig. 46.)

BLOOD SERUM.

(Figs. 24 and 25.) On plain blood serum, human and beef, with or without the addition of two per cent of glycerine, and on Loeffler's alkaline blood serum the same general differences were noted as on the other media.

The bovine bacillus measured between one and two microns; the human bacillus between two and three microns; both varieties generally stained evenly and deeply. Thus the length averaged less than on glycerine agar and longer than on Dorset's egg medium.

BOUILLON.

(Plate IX.) All bouillon used was six per cent glycerine, veal broth, peptone bouillon one and two-tenths per cent acid to phenolphthalein.

The macroscopic differences of the two varieties were rather more apparent here than on other media.

The human cultures yielded thicker, more profuse, and more wrinkled growth, sooner acquiring a dark cream color.

The bovine cultures, though covering the whole surface and pushing up the sides of the flask, did not form such a dense membrane, nor become so wrinkled and colored.

Microscopically, we found that both cultures yielded relatively long forms on bouillon. The human bacillus showed here also a greater variation than the bovine bacillus, branched bacilli being not uncommon. The length of the human

bacillus varied from two and three-tenths to four and nine-tenths microns. In the bovine cultures branched bacilli were not found. The length varied from two to three and five-tenths microns as a rule — occasional very short deeply-stained bacilli were found.

Both the human and bovine cultures showed much variation in staining. Irregularly-stained rods and faintly-stained rods containing more deeply-stained oval or round bodies were very common.

Two flasks, one inoculated with the bovine culture, the other with a human culture in which the inoculation masses had fallen to the bottom and there slightly increased in size, showed at the end of ten and eleven months respectively great numbers of branched bacilli. The mass of human bacilli was almost wholly made up of branched bacilli which stained deeply and solidly, showing none of the spore-like bodies and barred bacilli found with branched bacilli occurring under more favorable conditions of growth. The globule of bovine bacilli did not show so many branched bacilli, though these were quite numerous. The majority of bacilli were long and slender, and showed some irregularity in staining.

BRAIN.

On this medium multiplication is rapid, the colonies becoming visible in six or eight days. The growth spreads over the surface rapidly, forming in four to six weeks a thick layer, which at the bottom is moist and white, while at the top it is slightly dryer and of a darker brownish pink tint. As on the other media, the human cultures grow more luxuriantly, though the first growth occurs simultaneously with both. The growth separates easily from the brain in layers. It is generally thrown up into convolutions, formed by the bowing of the expanding growth, but it does not acquire the hardness found in old glycerine agar cultures. The bovine cultures under cultivation for eleven months have not shown the brownish coloration of the human culture, and retain a more moist appearance and are of softer consistency.

The changes in morphology produced by cultivation on brain is a most striking one. The forms about to be described occurred within the first two to three weeks, with all of the eleven cultures used.

The human cultures showed, as on the other media, greater variation than the bovine. The average length of the human bacilli ranged from seven to fourteen microns. Branched bacilli occur in great numbers, and are found in every field in cover-glass preparations.

There is a great variation in staining. Some bacilli stain deeply, some lightly. The greater number stain irregularly, and the round and oval spore-like bodies occur in greatest abundance. Many rods exhibit the simple irregularity in staining found in sputum bacilli, while other rods are made up of alternate stained and unstained segments, surrounded by a very definite enclosing membrane. Again, we find in large numbers very long, thread-like forms made up of alternate faintly and deeply stained segments, the latter often oval in shape and of greater length than the fainter stained part, thus giving the appearance of a chain of short bacilli. Often these curiously segmented thread-like forms contain one or more round, intensely-stained bodies, usually of slightly greater diameter than the rest of the rod, often situated eccentrically, as if they were being extruded from the bacillus. The small and intensely-stained bodies are found most often in faintly-stained, otherwise homogeneous rods, occasionally in branched bacilli, and also free in the preparations.

Besides the small, deeply-stained bodies just described, there were found other larger, equally deeply-stained bodies, often several times the diameter of an ordinary bacillus. These were round or more often oval in shape, and were not the clubbed swellings and other irregularities so often described, but were definite, shortly outlined bodies. They were occasionally found lying free in the preparations.

Rarely, in the human cultures, exceedingly large, round bodies several microns in diameter were found in branched bacilli at the point of junction of the three branches; still

more rarely the center of this body was occupied by a round, unstained refractive body, such as has been described by Fischel⁵ in the club swellings of the avian bacillus.

With the exception of the last described forms, which undoubtedly are degenerate, all those found in the human cultures were found in the bovine cultures, although with less frequency. Branched bacilli and the thread-like rods were found, while the small and large deeply-staining spore-like bodies were found in as great abundance as in the human cultures. In the cultures isolated by ourselves the average lengths were less than in the human cultures, rarely exceeding six to eight microns in length. In the bovine culture from Kral exceedingly long thread-like forms were found.

Remarkable as the changes described seem when we compare the two extremes, one the short rods averaging about one micron in length occurring on egg, the other the long filiform and branched bacilli ten to fifteen microns long occurring on brain, yet they are constant in their occurrence, and one extreme may be changed into the other at once by change of medium.

SUMMARY.

1. The tubercle bacillus undergoes marked changes in morphology with change of culture medium.

2. The microscopic characteristics of a fully developed culture are fairly constant for each medium.

3. Growth for several generations on a given medium has not tended to impart fixed characteristics, the change in form being just as prompt and complete as when transferred after a single generation.

4. These changes cannot be explained by assuming that the sole difference is in the favorability of the medium for the growth of the tubercle bacillus. Both Dorset's egg medium and the brain medium must be classed as extremely favorable ones, growth on each appears at about the same time and progresses about equally rapidly. The reaction of the medium also does not explain these changes, as the different media may have precisely the same reaction and yet these changes occur.

DISCUSSION OF THE FORMS DESCRIBED.

Ever since the discovery of the tubercle bacillus there has been much discussion concerning the significance of the deeply-stained spore-like bodies found in sputum and in pure cultures. They have been regarded as spores or "resting bodies" by most of those describing them.

McWeeney⁶ described these bodies in sputum bacilli and also as occurring free in sputum. He stated that in the search for tubercle bacilli the presence of these bodies may be taken as evidence of the presence of tubercle bacilli.

W. H. Smith⁷ shows photomicrographs of similar bodies in branched bacilli found in sputum.

Marpmann⁸ also described these bodies, as well as branched bacilli, in sputum.

In pure cultures they have been described by Nocard and Roux⁹ in avian cultures; by Metchnikoff¹⁰ in cultures virulent for rabbits; by Klein¹¹ in cultures (source not stated) when grown beneath the surface in alkaline bouillon; by Hayo Bruns¹² in cultures five to six months old on glycerine agar at 37° C.; by Fischel⁵ in avian and mammalian bacilli grown either at abnormal temperatures or on media to which antiseptic substances had been added; by Coppen-Jones,¹³ who described these bodies in great detail, in cultures grown on glycerine agar for three to four months; by Semmer¹⁴ in old cultures grown on potato at a temperature varying from 25–35° C.; by Mafucci¹⁵ in avian cultures grown at high temperatures; by Strauss¹⁶ also in avian cultures at high temperatures; by DeSchweinitz and Dorset¹⁷ in acid-containing media. They state that the adaptability of the tubercle bacillus to a variety of media can best be explained by spore formation. By V. Babes¹⁸ in old cultures both of mammalian and avian origin; by Theobald Smith⁴ in human and bovine cultures grown at 37–38° C. on dog's serum.

In the present work we have found the smaller of the deeply-stained bodies described above present in great numbers during the second week of growth on Dorset's egg medium and on brain. They are to be found in fewer

numbers in cultures on all ordinary media. They were found in great numbers in a twelve days' old bovine culture (4) on glycerine agar, and also on the water of condensation of a four months' old culture of bovine 2 on two per cent glycerine beef serum.

It cannot be said that these bodies are due to physical agents acting during the process of making cover-glass preparations. Heat we have done away with by our method of fixing with formalin. Osmosis could only produce irregular shrinkings of the protoplasm, but not these perfectly definite, sharply contoured bodies.*

That they are not degeneration products is shown by their constant occurrence in young, rapidly-growing cultures.

As described in the first part of this paper, the cultures on Dorset's egg medium show these bodies varying in size from a small, round object at one end or in the middle of the rod to an oval, bacillus-like body occupying nearly the whole of the rod. Occasionally two such bodies are found in the same faintly-stained rod as if division had occurred. The resemblance of these oval bodies to the characteristic bacillus of the older colonies has been pointed out.

On brain, owing to the greater size of the bacilli, these bodies can be better studied. They are best seen in bovine cultures. Their function cannot be absolutely determined. They have been proven to be no more resistant to heat than the bacilli themselves. That these bodies are in some way concerned in the multiplication of the tubercle bacillus is suggested by their presence in all lengths in a single field of the microscope, while some are found apparently in the act of division. The drawing (Fig. 50) was made from the same preparation as the photomicrograph (Fig. 47), in which all forms drawn can be found.

We are inclined to believe that these bodies are true resting bodies which by growth and division yield new individuals, and that this process may occur within the degenerated bacillus itself.

* Experimental Morphology, Davenport.

BRANCHED BACILLI.

It is largely on the occurrence of branched and filiform bacilli that the claim of pleomorphism and classification of the tubercle bacillus among the higher bacteria has been made.

Nocard and Roux⁹ and Mafucci¹⁵ described branched bacilli in avian cultures. Metchnikoff¹⁰ obtained branched bacilli in avian cultures and possibly in mammalian cultures accustomed to growth at high temperatures (43.6° C.).

Klein,¹¹ in growth secured below the surface in bouillon, found exceedingly long and branched bacilli, but he did not state the source of his cultures.

Fischel⁵ obtained branched and filiform bacilli in avian and mammalian cultures grown at 40° C. in media to which mildly antiseptic substances, as boric acid and thymol, had been added.

Dixon¹⁹ described cultures virulent for guinea-pigs, constantly yielding branched forms when grown at 40° C., on glycerine agar.

Coppen-Jones¹⁸ by maceration and by section of cultures grown on glycerine agar found constantly branched and filiform bacilli in cultures only three to four weeks old. He found these forms only on the surface of the cultures and came to the conclusion that a free supply of oxygen is necessary for their occurrence.

Hayo-Bruns¹² found two cultures of human origin (out of a great number examined) which at the age of five to six months showed many branched and filiform bacilli. His cultures were grown on glycerine agar at 37–38° C.

Lubinski²⁰ on acid potato media obtained in the first few days of growth extremely long filiform bacilli. At the end of three to four weeks his cultures consisted nearly wholly of these thread-like forms which did not show branching. The cultures used were probably mammalian.

Semmer¹⁴ in old potato cultures grown at temperatures varying from 25–35° C. found branched and filiform bacilli which were much thicker than the ordinary tubercle bacilli.

V. Babes¹⁸ has described branched filiform and clubbed bacilli in tissue and in bouillon cultures of the avian bacillus.

Lubarsch²¹ found branched and filiform bacilli in old cultures of the avian bacillus on glycerine agar.

Dorset²² reported branched bacilli in a six weeks' old culture (four weeks in a thermostat and two weeks at room temperature) which had become contaminated with a streptothrix.

L. M. Loeb²³ found branched and filiform bacilli fourteen to twenty-nine and four-tenths microns long in bouillon cultures grown at 37–39° C., two weeks old, during which time the bouillon was allowed to evaporate to nearly one-half its original volume. These cultures were originally virulent for guinea-pigs. Sub-cultures could not be obtained.

Thus a review of the work reported upon this subject shows that, in the majority of instances, the branched and thread-like forms were found in avian cultures. A few observers stated neither the source nor virulence of the cultures used. Of those using mammalian varieties, the majority placed the cultures under abnormal conditions using unfavorable temperatures or media. In no instance is it definitely stated that the bovine variety produced these long and branched forms.

In sputum, branched bacilli have been reported by Czaplewski,²⁴ McWeeney,⁶ Marpmann,⁸ Craig,²⁵ and W. H. Smith.⁷

In our own work we have found branched bacilli occasionally in very young bovine cultures (five to eight days) on Dorset's egg medium. We have frequently found them in bouillon cultures six to twelve weeks old of the human bacillus, younger bouillon cultures not being examined, as these examinations were done to ascertain the purity of the cultures before concentrating for tuberculin. On brain, in young cultures, two to three weeks old, both human and bovine, we have constantly found branched bacilli in large numbers. In two instances — once with a bovine, once with a human culture — we have found branched bacilli in the slight growth occurring during many months at the bottom of the

flasks of bouillon. These last differed somewhat from the other branched forms found in rapidly growing cultures in that they stained more evenly and deeply, resembling those illustrated by M. Dorset²⁷ and L. M. Loeb.²⁸

SUMMARY.

1. The greatest variations in form and staining reaction are found in rapidly growing cultures, and we agree with Copen-Jones in regard to the conditions best suited for the production of branched and filiform forms, namely, a favorable medium and free access to oxygen.

2. The only interpretation of the great diversity of form assumed by the tubercle bacillus when grown under the most favorable conditions is that it is truly pleomorphic and should be classed among the higher bacteria.

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DESCRIPTION OF PLATES.

PLATE IV.

- FIGS. 1 and 2. Bovine culture 2. Seven days' growth. Show length of bacilli, arrangement in pairs, and difference in stain. X 1,000.
- FIG. 3. Same culture, sixteen days' growth. Shows coccus forms and deeply-stained bodies in faintly-defined bacilli. X 1,000.
- FIG. 4. Same culture, twenty-three days' growth. X 1,000.
- FIG. 5. Same culture, forty-six days' growth. X 1,000.

PLATE V.

- FIG. 6. Emulsion of bovine culture 7. Preparation made before inoculating tubes from which the following five preparations were made. X 1,000.
- FIG. 7. Same culture, six days' growth. X 1,000.
- FIG. 8. Same culture, eight days' growth. X 1,000.
- FIG. 9. Same culture, eleven days' growth. X 1,000.
- FIG. 10. Same culture, fourteen days' growth. X 1,000.
- FIG. 11. Same culture, eighteen days' growth. X 1,000.

PLATE VI.

- FIG. 12. Human culture 1, seven days' growth. X 1,000.
- FIG. 13. Same culture, sixteen days' growth. X 1,000.
- FIG. 14. Same culture, sixty-eight days' growth. X 1,000.
- FIG. 15. Human culture 3, six days' growth. X 1,000.
- FIG. 16. Same culture, sixteen days' growth. X 1,000.
- FIG. 17. Same culture, fifty-two days' growth. X 1,000.

PLATE VII.

- FIG. 18. Human culture 1, eight weeks' old culture on Dorset's egg medium. X 1,000.
- FIG. 19. Human culture 3, four weeks' old culture on Dorset's egg medium. X 1,000.
- FIG. 20. Bovine culture 5, thirteen weeks' old culture on Dorset's egg medium. X 1,000.
- FIG. 21. Bovine culture 2, fourteen weeks' old culture on Dorset's egg medium. X 1,000.

FIG. 22. Human culture 3, seven weeks' old culture on glycerine agar. X 1,000.

FIG. 23. Bovine culture 2, twelve weeks' growth on glycerine agar. X 1,000.

PLATE VIII.

FIG. 24. Human culture 1, eight weeks' old culture on human blood serum. X 1,000.

FIG. 25. Bovine culture 2, ten weeks' old culture on beef serum. X 1,000.

FIGS. 26 and 27. Bovine culture 2, four weeks' growth on brain. X 1,000.

FIGS. 28 to 33 inclusive. Human culture 1, seven weeks' growth on brain. X 1,000.

PLATE IX.

FIG. 34. Human culture 1, eleven weeks' growth on bouillon. X 1,000.

FIG. 35. Bovine culture 2, six weeks' growth on bouillon. X 1,000.

FIG. 36. Human culture 1, and bovine culture 2, forty-five days' growth on bouillon in 250 cc. flasks. Reduced from a water color.

FIG. 37. Human culture 1, growth at bottom of flask. X 1,000.

FIG. 38. Bovine culture 2, growth at bottom of flask. X 1,000.

PLATE X.

FIGS. 39, 40, and 42. Human culture 1, seven weeks' growth on brain. X 1,000.

FIGS. 41 and 43. Human culture 3, eighteen days' growth on brain. Note the spore-like bodies lying free in preparation. X 2,000.

PLATE XI.

FIG. 44. Bovine culture 3, twenty days' growth on brain. Note spore-like bodies of varying sizes within the bacilli and free in preparation. X 2,000.

FIG. 45. Bovine culture 2, growth on water of condensation of eleven weeks' old culture on blood serum, shows deeply-stained bodies. X 1,000.

FIG. 46. Bovine culture 6, twelve days' growth on glycerine agar, shows deeply-stained bodies. X 1,000.

FIG. 47. Bovine culture 5, eleven days' growth on brain. Note deeply-stained bodies of all sizes in stained bacilli. X 2,000.

PLATE XII.

FIG. 49. Drawing from a four days' old culture on egg of bovine 3.

FIG. 50. Drawing from bovine culture 5. Same preparation as Fig. 47.

FIG. 51. Human culture 1, on brain. Drawing from twenty days' old culture. Note deeply-stained bodies and eccentric arrangement in bacilli, otherwise irregularly stained.

PLATE XIII.

FIG. 52. Human culture 1, branched bacilli found in nine weeks' old colony on brain.

FIG. 53. Bovine culture 5, six days' old culture on egg. X 1,000.

FIG. 54. Bovine culture from Kral, ten days' growth on brain. X 2,000.

FIG. 55. Bovine culture 1, one year's growth at bottom of a flask of glycerine.

FIG. 56. Bovine culture 5, six weeks' growth on egg. Note long-beaded bacilli. X 1,000.

A HITHERTO UNDESCRIBED FIBRILLAR SUBSTANCE PRODUCED BY CONNECTIVE-TISSUE CELLS.*

F. B. MALLORY, M.D.

(*From the Sears Pathological Laboratory of the Harvard University Medical School.*)

By the aniline blue connective-tissue stain the ordinary connective-tissue fibrils are colored deep blue, while elastic tissue fibers, unless degenerated, appear colorless or pale pink. In addition to these two kinds of fibrils there is a third variety which is stained red by the same method. These red fibrils are easily overlooked unless they are numerous and deeply stained by the acid fuchsin, because the blue in the section overpowers them. Although I have known of the existence of these fibrils for over two years, it was only recently that I paid much attention to them or attempted to stain them differentially so that a true idea of their number and significance could be obtained.

The simplest and most satisfactory method of demonstrating them is as follows:

1. Fix in Zenker's fluid. The tissue should be as fresh as possible and cut into thin sections (2 to 4 mm. thick) for the best results.
2. Stain celloidin or paraffin sections in a one per cent aqueous solution of acid fuchsin over night in the cold, or better still, for twenty to thirty minutes in the paraffin oven (56° C.).
3. Wash quickly in water (not over two to five seconds). Water extracts acid fuchsin very rapidly.
4. Differentiate in a one-fourth per cent aqueous solution of permanganate of potassium for twenty to forty seconds. This step must not be prolonged beyond the exact time needed, or the section will be decolorized.
5. Wash quickly in water (not over two to five seconds).
6. Dehydrate in alcohol.

* Read at the meeting of the American Association of Pathologists and Bacteriologists in Washington, May 14, 1903. Received for publication Oct. 14, 1903.

7. Clear in oleum origani cretici or in xylol.

8. Mount in xylol balsam.

Results almost equally as good can be obtained by staining sections in a cold one per cent solution of acid fuchsin for five to twenty minutes and differentiating in a very dilute solution of permanganate of potassium for about thirty seconds.

While not an absolutely differential stain for these fibrils, the method, for the most part at least, is perfectly satisfactory. It stains intensely red not only these fibrils and the cell nuclei, but also fibrin, the contractile elements of striated muscle cells, the coarse, differentially staining fibrils of smooth-muscle cells, neuroglia fibers, and the cuticular surfaces of epithelial cells. Ordinary connective-tissue fibrils appear from brownish yellow to colorless; elastic fibrils, unless degenerated, are bright yellow.

These red-staining fibrils are sometimes brought out with great distinctness of a deep pink color on an almost colorless background by the eosin methylene blue method, after fixation in Zenker's fluid. They can also be stained fairly well after the same fixation by the phosphotungstic acid hematein method. Sometimes the aniline blue connective tissue stain is preferable for their demonstration, more especially in the embryo, but when used for this purpose the red stain should be made intense, the staining with aniline blue should not be prolonged, and the sections should be as thin as possible.

This staining method slightly modified from the directions as originally published is as follows:

1. Fix in Zenker's fluid.
2. Stain paraffin sections in a one per cent aqueous solution of acid fuchsin for five to twenty minutes.
3. Wash quickly in water (not over five seconds).
4. Place in a one per cent aqueous solution of phosphomolybdic acid for five minutes or longer.
5. Wash quickly in water (not over five seconds).
6. Stain in the following aniline blue mixture for one to five minutes:

Aniline blue soluble in water (Grübler)	0.5
Orange G (Grübler)	2.0
Oxalic acid	2.0
Water	100.0

7. Wash quickly in water (not over five seconds).
8. Wash thoroughly and dehydrate in several changes of alcohol.
9. Clear in xylol.
10. Mount in xylol balsam.

For ordinary purposes, when no attempt is made to bring out these red-staining fibrils, a better general tissue stain is obtained by placing the sections in a one-half per cent aqueous solution of acid fuchsin for five minutes only. For certain organs, such as the liver and the lymph nodes, it will often be found advisable to dilute the acid fuchsin still more.

The chief drawback of the methods given is that they do not differentiate between these red-staining fibrils of connective-tissue cells and the differentially staining fibrils of smooth muscle cells. In fact, these hitherto undescribed connective-tissue fibrils can be stained fairly well by the two methods generally used for the demonstration of the myoglia fibrils, namely, Heidenhain's iron hematoxylin stain and Benda's differential method. Moreover, they are often stained, to some extent at least, by the various differential stains (Mallory, Benda, Huber) for neuroglia fibrils, with the exception, apparently, of Weigert's. With his method the results were entirely negative in my hands.

These fibrils, as a rule, are not very common in normal tissues, except possibly in one situation to be spoken of later, and have to be hunted for with an oil immersion lens. I have made no particular search for them, but have noted them, among other places, in the dense fibrous tissue of the breast, the corium, and the pia of the spinal cord. They usually occur singly or in small clumps. In the embryo they make their appearance in certain regions where the connective tissue is dense, as around cartilage, at a comparatively early stage, that is, at least as early as the time when the smooth-muscle cells are acquiring their differentially staining fibrils.

The most interesting field for their study is in inflammatory tissue of all sorts, more especially where it is growing rapidly, as for example, in granulation tissue, in the stroma of carcinomata (especially the medullary carcinomata of the breast), and in other tumors in which connective tissue plays an important part. In other words, these cell fibrils follow closely the fate of the connective-tissue cells: they are numerous and prominent when the cells are abundant, active, and proliferating; they are hard to find when the cells are few in number and in an inactive state.

These fibrils which are stained red by the two principal methods used to demonstrate them appear round and solid; they usually run a straight or slightly curved course, but occasionally are wavy. They vary considerably in thickness, but are almost invariably considerably coarser than the individual elements of the other variety of connective-tissue fibrils. Sometimes they appear quite coarse, but these larger fibrils seem to be formed of bundles of finer ones. They rarely show any of the sharp bends seen in neuroglia fibrils in stained sections, but where the cut ends project above the surface of the section they are often bent around like fish hooks. These fibrils are further characterized by being refractile, acidophilous, and of indefinite length, and by not branching.

They bear the same relation to the cell protoplasm that neuroglia fibrils do; that is, they touch or run through the outer surface of the protoplasm and continue indefinitely in two directions; one end does not start in the cell. The fibrils from one cell apparently run to other cells, so that they are all more or less connected together.

These red-staining fibrils, which on account of their intimate relation to the cells may well be called (after the analogy of neuroglia and myoglia fibrils) fibroglia fibrils, to distinguish them from the blue-staining, intercellular variety, emphasize the fact that in many situations connective-tissue cells are flat cells with flat oval nuclei, and, with their red-staining fibrils, lie between layers of the blue-staining fibrils. When the cells are examined as they lie flat in

the section it will be seen that the cell-fibrils tend to run in the direction of the long axis of the nucleus, but that they spread out in a more or less fan-shaped manner as they get away from the cell. If the protoplasm has processes extending out in three or more directions from the cell the fibrils will spread in the same manner. Where the cells are at all numerous the fibrils run in all directions in parallel planes and consequently present a very confusing picture. When the cells are seen from the side, the nuclei appear spindle-shaped and the cell-fibrils show no tendency to spread. When they are cut across, the cell-fibrils can often be seen to run in clefts between masses of the intercellular fibrils. When, as in very young granulation tissue and in spindle cell sarcomata, for example, the cells are spindle-shaped, the cell-fibrils show little or no tendency to spread. Under these conditions a connective-tissue cell may closely resemble a smooth-muscle cell. In inflammatory processes involving smooth-muscle tissue it is sometimes impossible to tell to which variety of cell certain fibrils belong.

The chief interest in these fibrils in normal tissues lies in their relation to the basement membranes of various glands Mall* has recently called attention to the presence in renal tubules of a true basement membrane inside of what has hitherto been regarded as the basement membrane. The latter is composed of white, fibrous tissue and of reticulum. According to Mall the true basement membrane has entirely different chemical properties from these tissues. By means of maceration of renal tissue in bicarbonate of soda for several days he was able to demonstrate it as a homogeneous tube lying next to the lining epithelial cells. The membrane could not be stained by Weigert's elastic tissue stain, and was not stained blue by the aniline blue method. Chemically it reacted much like the membranes of elastic fibers.

The epithelium of the coil glands of the skin and of the ducts and glands of the breast rests on a layer of cells and fibers which are regarded by many to be more or less altered,

* Note on the Basement Membranes of the Tubules of the Kidney. *Bulletin of the Johns Hopkins Hospital*, 1901, xii, 133.

smooth-muscle cells. Benda * goes so far as to regard these cells as smooth-muscle cells of epithelial origin, owing to their situation inside of what has ordinarily been regarded as the basement membrane.

Sections of the kidney stained by the first method given in this paper show that the epithelial cells of the tubules rest on a thin layer of very fine fibrils which run parallel to each other lengthwise of the tubules, and which are stained intensely red by the method employed. The fibrils, without much question, form a part, perhaps the whole, of the homogeneous tube described by Mall as the true basement membrane of the renal tubules.

In the coil glands of the skin and in the glands of the breast the epithelial cells likewise rest on red-staining fibrils which are more numerous and often much coarser than those in the kidney. In the ducts of the mammary gland the fibrils are usually still more abundant. Although the fibrils commonly run lengthwise of these glands and ducts, they can sometimes be found running around them. They also often run between the epithelial cells and may reach almost to the lumen. The cells which go with these fibrils often form quite a distinct layer outside of the lining epithelium.

In arteries and in at least the larger veins the endothelial cells rest on an abundant layer of red-staining fibrils which run in a parallel layer lengthwise of the vessels.

The point which now remains to be determined is the nature of the cells which produce the fibrils on which the cells in the glands and in the blood vessels rest. As already stated, the methods here given do not differentiate between the red-staining fibrils of connective-tissue cells and the coarse fibrils of smooth-muscle cells. The two kinds of cells are so closely related that it is possible that their differentially staining fibrils are alike. There is, however, a certain amount of evidence which can be brought forward in favor of the view that these fibrils lining blood vessels and certain glands are the product of connective-tissue cells.

* Das Verhältniss der Milchdrüse zu den Hautdrüsen. Dermatol. Zeitschr., 1893-94, i, 94-110.

In the first place, it is probable that the fibrils in the various glands in which they occur and in the blood vessels are all produced by the same kind of a cell, but it is difficult to conceive of the fibrils which are spread out in so thin a layer around the tubules of the kidney as belonging to smooth-muscle cells.

More important than this, however, is the fact that in all glandular growths of the breast these fibrils are produced in the greatest abundance and occupy the same situation adjoining the lining epithelium. Another point is that in carcinomata the epithelial cells show many of the red-staining fibrils in close contact with them, and these fibrils we know to be produced by connective-tissue cells.

It is not easy to suggest the significance of these red-staining fibrils. It is hardly conceivable that they can give rise to the blue-staining variety by splitting or by diminishing in caliber, and by changing in chemical properties. Their presence in such intimate relation to the protoplasm of the cells is, perhaps, an argument in favor of the view that the blue-staining fibrils are intercellular in origin and not a cast-off product of the cytoplasm.

If the fibrils existed only in connective tissue it might be thought, in consequence of their extending apparently from cell to cell, that they had something to do with the relation of the cells to each other, but their presence in large numbers in the true basement membrane of various glands and beneath the endothelium of blood vessels is against this view, and favors the idea that they have elastic or contractile properties.

The demonstration of their existence opens up a new and interesting line of research work.

SUMMARY.

Connective-tissue cells produce, in addition to elastic fibers and the ordinary intercellular fibrils, a third variety of fibrils (fibroglia fibrils) which differ from them chemically and morphologically and which have apparently the same staining properties as the coarse, differentially staining (myoglia)

fibrils of smooth-muscle cells. The fibroglia fibrils bear the same relation to the connective-tissue cells that neuroglia fibrils bear to neuroglia cells. They are present in great numbers in all actively growing connective tissue, both of inflammatory and of tumor origin. They are scarce in normal tissues except, perhaps, in certain situations; they apparently form the true basement membrane of the tubules of the kidney, of the coil glands of the skin, and of the glands and ducts of the breast. They also occur in abundance beneath the endothelium lining arteries and the larger veins.

DESCRIPTION OF PLATE XIV.

FIG. 1. — Connective tissue stroma of a medullary carcinoma of the breast showing the arrangement of the red-staining (fibroglia) fibrils with reference to the nuclei when the cells are seen lying flat.

FIG. 2. — An alveolus from a carcinoma of the breast showing the arrangement of the fibroglia fibrils with reference to the epithelial cells.

FIG. 3. — Cross section of a gland from an intracanalicular papillary fibroma of the breast. The fibroglia fibrils are cut transversely and form a more or less complete layer between the lining epithelium and the broad hyaline membrane which surrounds the gland.

FIG. 4. — Oblique section of a small artery showing the longitudinally running fibroglia fibrils on which the endothelium rests.

ON THE APPEARANCE AND SIGNIFICANCE OF CERTAIN
GRANULES IN THE ERYTHROCYTES OF MAN.*

VICTOR C. VAUGHAN, JR., A.B., M.D.

There is probably no one element the examination of which has more frequently led the microscopist to erroneous conclusions than the red blood cell of man. The varied appearances which have been seen in it as the result of various methods of examination have led to the most diverse interpretations of their meaning and the importance which should be attached to their occurrence from a clinical point of view. That such should be the case appears to be only natural when we consider that it is so very sensitive to extraneous influences, since it is not only one of the most highly differentiated of all cells, but also is lacking in a nucleus, that portion of the cell without which it is generally agreed that no constructive metabolism can take place. For this reason the manifold retrograde alterations which occur in it as the result of even slight variations from normal conditions have caused the true changes which take place during its life history to be entangled in a network of doubtful and deceitful appearances, from which it is well-nigh impossible to separate them.

The normal fully developed erythrocyte found in the circulating blood of man may be defined as a bi-concave cell of homogeneous appearance, with an opaque yellowish rim and lighter central portion, and containing no nucleus. Authorities are generally agreed upon the fact that this cell is developed from the nucleated hemoglobin-containing cells of the bone marrow, the normoblast and the megaloblast, the latter chiefly during embryonic life, by the loss of the nucleus. They differ, however, as to the manner in which the nucleus is lost, some holding that it is extruded en masse, while the majority, especially of the later writers, are in favor of an intracellular disappearance of the nucleus.

* Received for publication Aug. 12, 1903.

Kölliker in 1846 first upheld the view that the nucleus gradually disappears within the red cell. He was able to find in the embryonal liver all transition stages between erythrocytes with well-preserved nuclei and others containing only one or two small, round or cornered granules. This view as to the fate of the nucleus has been subsequently upheld by Neumann, Löwit, Foa, Osler, Mondino, Sala, Schmidt, and Spuler. More recently, Israel and Pappenheim have carried on investigations with the fresh blood of mouse embryos, in specimens of which they were unable to find free nuclei, whereas they found both normoblasts and megablasts with gradually fading nuclei, evidently disappearing through a process of karyolysis. They were able to notice the same changes in fixed specimens and account for the appearance of free nuclei, either by the supposition that they are mechanically dragged out of the cell by the usual method of making smears, or that there has been a shrinking away of the protoplasm of the red cell around the nucleus. Further evidence in favor of the intracellular disappearance of the nucleus has been furnished by the researches of Wooldridge, Lilienfeld, Botazzi, and Capelli, who, by chemical analysis, have been able to demonstrate the presence of nuclear material within the fully developed normal erythrocyte.

Rindfleisch in 1880 first advanced the theory of the extrusion of the nucleus, which view later met with confirmation through the work of Howell, who described cells in which the nucleus was fixed in the act of extrusion. He further makes mention of a cell of this nature in which granules were at the same time found diffusely scattered through the cell protoplasm. These Howell thinks represent particles of chromatin thrown off from the nucleus before its final extrusion. More recently Maximow has also spoken in favor of this view. Still other observers, as Malessez, Fellner, and Duval, think that the nucleated red cell extrudes a portion of its substance in the form of a red disc entirely free from nuclear material, thus forming the erythrocyte; while Ehrlich believes that the nucleus of the normoblast is lost by extrusion, while that of the megaloblast disappears within the cell.

This doubt as to the morphological changes which occur during the transition of the red cell from its nucleated to the unnucleated state has, it is safe to say, been accountable for a great part of the discussion which has arisen with regard to the clinical significance of many of the changes noted in the erythrocytes in health and disease, and it was with the view of possibly throwing some little additional light upon this subject that the following investigations were undertaken.

In the study of a structure which is so easily influenced by extraneous factors as the erythrocyte, the method of examination is a matter of paramount importance, and our attempt should always be to obtain the red cell in as nearly a normal state as possible. Since the introduction by Ehrlich of the method of making permanent specimens through the employment of some agent which fixes the blood elements, the examination of the fresh blood has been sadly neglected, and it is probable that some valuable information connected with the life history of the red cell has been overlooked through this omission. Erb, who in 1865 examined the unstained fresh blood of normal dogs and rabbits, saw red cells containing granules which were especially well brought out after treatment with dilute acetic acid, and which he thought represented a transition form between the polymorphonuclear leucocytes and the red blood cell. He found the number of these granular cells distinctly increased after hemorrhage, and decreased as a result of starvation. He was furthermore able to find them in the normal blood of man in scanty numbers, while in that of the newly born they were present in a large proportion of all cells. They were also found to increase in number in recovery from severe hemorrhage occurring in connection with gastric ulcer or following an abortion. These granules were of different sizes, and Erb thought that they represented the remains of the nucleus of a polymorphonuclear leucocyte, and considered their appearance as a valuable sign of blood regeneration.

Recently, since the introduction of the method of staining the fresh unfixed blood with neutral red, peculiar changes occurring in the red cell have been noted by different

observers and variously interpreted. In 1896 Israel and Pappenheim noticed granules in the erythrocytes stained with neutral red, which they considered to be artifacts, since they were irregular in shape and were present in great numbers in some cells, while in the majority they were wholly lacking. In 1898 Müller described certain granular changes occurring in the red cells which could be well brought out through the action of neutral red, and which he considered as representing a phenomenon occurring in connection with coagulation. These granules could be found throughout certain cells, or arranged around the periphery. Occasionally they were found entirely outside of the erythrocytes. They were, for the greater part, of small size and often appeared bound together by delicate threads. Schwalbe in 1899, on examining the blood of a pigeon stained by allowing a crystal of neutral red to dissolve in the serum, obtained a picture corresponding very closely to that seen in Figure 1 (Plate XV.). He has been able to watch the separation of little particles from the nucleus, and thinks with Müller that the change seen represents a wandering out of the nuclear substance occurring in connection with the process of coagulation. Schwalbe and Solley in 1902, on examining fresh blood to which neutral red and methyl violet had been added, noticed many red cells giving off separation products, which stained more or less intensely with methyl violet and resembled in all respects the preëxisting blood plates. They state that after poisoning with toluendiamin, nearly all of the erythrocytes are filled with blue granules, resembling those which Heinz found as the result of the action of many poisons, and Bloch observed in cases of poisoning with pyridin. The authors state that the same granules staining blue with methyl violet appear in normal blood during the process of coagulation, the difference in the appearance of the poisoned blood being simply a quantitative and not a qualitative one. They furthermore state that they have seen basophilic granules in the blood of normal guinea-pigs and rabbits, and arrive at the conclusion that those granules seen in fresh specimens have to do solely with the formation of blood plates and coagulation, while

those seen in both fresh and stained preparations may be due to changes in the hemoglobin content, allowing the granular structure of the erythrocyte to show through.

Maximow, in 1889, speaks of granules as occurring in young erythrocytes, and staining with neutral red, which, however, he claims have nothing whatever to do with the nucleus, but first make their appearance within the cell around its periphery as the nucleus is undergoing extrusion. He thinks that they stand in relation to the "nucleoid," which is developed within the red cell as the nucleus is extruded, and which is capable of demonstration only in fixed specimens. These granules, at first making their appearance at the periphery, gradually approach the central portion of the cell, and disappear as the development advances further, leaving the completely formed, normal, unnucleated erythrocyte.

Being myself firmly convinced that the only rational method of studying the life processes of the blood elements is to study them in as nearly a normal state as possible, most of this work has been confined to the examination of specimens of fresh blood, stained with polychrome methylene blue, and not subjected to the various alterations which are caused by all methods of fixation, either chemical or physical. The method of procedure has been as follows: The ear of the patient whose blood we wish to examine is thoroughly cleansed of all dirt particles by washing with alcohol. The blood is then drawn, the first drop being used as an index as to whether a free flow has been obtained or not. This is then wiped off with a clean towel, and a drop of the previously filtered stain placed over the site of puncture by means of a clean glass rod or pipette. The blood flowing from the wound thus mixes directly with the stain without coming in contact with the air, and the small drop thus obtained is immediately collected on a coverslip and placed at once upon a slide, where, if the drop is not too large and the slide and cover are clean, it spreads out into a thin film and may be examined at once. In order that the conditions

might be as little altered from the normal as possible, warm slides and covers and a warm stage were at first used, the cover-glass being rimmed with paraffin, but as it soon became evident that such precautions had little influence upon the appearance of the specimen, they were discarded as unnecessary. The examinations were in all cases made as soon as possible after the withdrawal of the blood. The stain used was in almost all instances Unna's polychrome methylene blue, as prepared by Grüber. It was used in undiluted form, and the best results were obtained when the amount of stain was small as compared to the size of the drop of blood. It may be said that the solution used was not isotonic with the blood serum, but the effect on the size and shape of the red cells, one of the most delicate tests of the tonicity of a fluid, when mixed in right proportion, was practically nil, the red cells showing neither evidence of crenation nor of swelling, in the majority of cases. In those instances in which changes of this nature were noticed, the specimen was immediately discarded and a fresh one made.

On examining a spread prepared in the above manner, it will be seen at the end of a few seconds that, although a majority of the red cells show no affinity whatever for the stain, having simply the appearance of normal erythrocytes in an unstained preparation, here and there, throughout the specimen, will be found a cell which contains more or less numerous granules, staining a decided reddish purple, having evidently shown a special affinity for methylene azure, the chromatin staining principle of the polychrome methylene blue. The cells showing this peculiar granulation are not numerous in normal blood, there being seldom more than two or three such seen in a field containing from two to three hundred blood cells, while in many fields they will be wholly wanting. The granules are usually found in cells whose hemoglobin content seems to be normal, and are not more numerous in cells showing endoglobular degeneration, crenation, and other evidence of departure from the normal. In fact, they seem to be less numerous, if anything, in these cells than in those in which the hemoglobin color and the

cell outline speak for a normal condition. The above-mentioned granules are at first of a decidedly pinkish appearance, which after the lapse of some time changes through a purple to a pronounced bluish tinge. They are of the most diverse sizes, some of them being quite large, while others are so small as to at first escape notice, were it not for the fact that they stand out so distinctly against the color of the hemoglobin. In normal blood, as mentioned above, the cells containing these granules are comparatively few in number, and the granules are, without exception, either scattered uniformly throughout the cell (Fig. 5 *c*), or arranged perhaps around the periphery (Fig. 5 *f*). Often an appearance such as that seen in Fig. 2 *c* or Fig. 3 *d* will be found, the granules being arranged in a line running directly across the entire diameter of the cell. When this is the case the granules are invariably of the smaller variety. Besides differences in size, there are also great differences in the shape of the granules, some of them being perfectly round, others being oblong, while still others are of the most diverse shapes. In many instances one can see that the granules are apparently connected with each other by very delicate thread-like strands which stain for the greater part as do the granules.

On examining the blood of a patient suffering from pernicious anemia, I was at once impressed by the enormous increase in the number of granular cells, and in order to make sure that this increase was a real rather than an apparent one, I have, in the various conditions in which I have studied the blood, counted from fifteen hundred to two thousand red cells and given the percentage of these which showed the granular appearance. Of course it must be understood at the outset that this percentage is of no value as an exact number, but is simply a convenient and trustworthy method of ascertaining whether or not the number of granular cells is or is not actually increased under certain conditions.

BLOOD OF APPARENTLY NORMAL INDIVIDUALS.

Case.	Number of red cells.	Per cent of Hb.	Per cent of all cells showing granules.
1 . .	4,800,000	90	0.8
2 . .	5,200,000	95	1.3
3 . .	5,000,000	95	0.9
4 . .	4,500,000	95	1.8
5 . .	5,100,000	90	1.3
6 . .	4,800,000	90	0.5
7 . .	5,500,000	95	0.6
8 . .	5,100,000	95	1.1

On examining the above table there is one fact which immediately strikes our attention, namely, the narrow limits between the percentage of cells showing granules in the blood of the various individuals studied. The constancy in this respect seems to be one of the most striking points in favor of the fact that here we are not dealing with an artifact or with cells showing degenerate changes. Moreover, if the latter were the case, we should expect to find the percentage of such cells increased on allowing the specimen to stand. Such, however, is not the case, and, after standing for several hours, the proportion of these cells does not seem to increase, nor do the granules in the individual cells show any augmentation in number. It must be admitted here, however, that after the lapse of some time the remnants of blood platelets and precipitates of the stain may settle down upon and around the red cells in such a manner that it is only by careful focusing that one can see that he has to deal, not with actual granules lying within the cell itself, but with foreign bodies resting upon it. Again, the fact that the number of granular cells does not increase after the lapse of sufficient time for the stain to act upon them, as can be well judged by the appearance of the leucocytes in a given field, the latter staining most beautifully even while in active ameboid motion, seems to be a point against the supposition that they represent changes in the blood preparatory to coagulation.

By the use of unfixed specimens we are enabled to observe the effect of different reagents upon these granules contained in the erythrocytes. For example, a preparation was made and examined in the usual manner. After the granules had taken the stain, a one-half per cent solution of acetic acid was allowed to flow under the edge of the cover-slip. On coming in contact with the reagent, the erythrocyte first becomes rather indistinct in appearance, as if it were out of focus, gradually losing its characteristic hemoglobin color, and ultimately disappearing from view. The granules stained reddish purple with polychrome, become changed to a decided blue on coming in contact with the acid. In cells containing these granules they are brought out with much greater distinctness as the remainder of the cell disappears. At first one may see masses of bluish-staining granules crossing the field, to all outward appearances free, but, judging from the fact that they keep so closely together, and the mass retains its form so well while moving, still held together within the disintegrating red cell. Finally the granules composing these masses begin to separate from each other, indicating that the red cell has been completely dissolved, and from this time they cannot be differentiated from the detritus and precipitates of the stain which may be present. The behavior of the nuclei of the leucocytes and of the blood platelets toward acetic acid is similar in all respects to that of the granules. Another specimen was prepared and stained in the same way and subjected to the action of hemolytic serum. The results obtained were practically identical with those of the preceding experiment. The granules were not dissolved by the action of the serum, but were apparently set free in the plasma, as the red cell containing them suffered hemolysis. Here again it is worthy of note that the employment of such serum has no hemolytic effect upon either the nuclei of nucleated red cells or upon the blood plates.

On the examination of an advanced case of pernicious anemia in which the number of nucleated cells seen in the circulating blood had been very abundant, it was found that fully one-third of all the erythrocytes seen showed the

presence of granules. The results obtained from the examination of the cases of pernicious anemia, with the exception of the above mentioned, in which it was unfortunately impossible to secure a count, are as follows:

Case.	Number red cells.	Per cent Hb.	No. nucleated reds seen in counting 500 leucocytes.	Per cent of all cells showing granules.
1 . . .	2,000,000	35	—	9.8
	1,820,000	32	32	10.1
2 . . .	1,660,000	32	37	10.4
	1,210,000	25	47	9.4
	885,000	22	29	11.1
	1,008,000	25	240	18.8
	974,000	27	12	7.7
3 . . .	3,500,000	78	20	10.0
	1,640,000	44	—	9.0

On comparing the results obtained in the above table with those in table one we see at once that the number of cells showing granules in the latter case is decidedly increased. More significant yet, however, are the changes which are noticed in the size and arrangement of the granules within the cells. Whereas, in the case of normal blood they were usually found more or less diffusely scattered throughout the cell, in that of pernicious anemia they are more apt to be massed together in the central portion (Fig. 5 *a*, *b*, *h*). Again, the size of the granules is, as a rule, much larger in the case of pernicious anemia. Case two is an exceptionally instructive one, as it shows the close relation which exists between the number of nucleated reds seen in the blood and the number of granular cells. During the first part of his attack both the number of nucleated reds and of granular cells was fairly constant. During the blood crises the number of granular cells increased enormously, and during his gradual failure, when the nucleated reds had fallen to twelve, the granular cells also reached their lowest per cent. An examination of this table at once led to the supposition that these granules probably stand in some

intimate relation with the nucleus of the red cell, and with this explanation in view I have tried to find other evidence to support the theory. If, indeed, the granules represent nuclear remains, where would we be more apt to find them than in the circulating blood of newly born infants, which, as we very well know, often contains nucleated red cells for a few days after birth? With the object of ascertaining, therefore, whether or not the percentage of granular cells was increased in the newly born, examination of infants' blood was made as soon as possible after birth, with the following results:

Case.	Percentage of all cells showing granules.
1. Child 16 hours old	3
2. Child 16 hours old	4
Child 6 days later9
3. Child 2 hours old	3
4. Premature child born alive at 7th month	7
Same child, after 4 days in incubator	3
5. Child 4 hours old	3.5
6. Child 3 hours old	4

In case two three normoblasts were seen, whereas in the rest, typical normoblasts were absent, as, although some of them were difficult to classify, no cell was considered a normoblast in which the nucleus had begun to show evidence of karyorrhexis. In all of these cases the arrangement of the granules was for the greater part in the central portion of the cell, as is well illustrated in Figure 2. In cases two and four some very instructive pictures were obtained. Many of the cells containing granules showed a very finely granular, centrally located, bluish-staining mass, around which, in the hemoglobin-containing portion of the cell or in some instances situated in this central mass or upon its periphery, could be found large, decidedly reddish purple, sometimes bright red, round homogeneous masses resembling nucleoli, and corresponding in all respects to the chromatin of the malarial parasite when stained in the same manner (Figs. 2 *e* and 5 *h*).

Similar bodies have been described by Howell, who states that they occur only when the process of blood production is markedly accelerated, and who looks upon their appearance as being due to a bit of the nucleus left adherent to the blood corpuscle after the escape of the former from the cell. They appear to correspond also very closely to the endoglobular bodies described in the blood of cats by Schmauch, and which he considers as representing the remains of the nucleus.

It is interesting to note with what rapidity after birth the blood of the infant assumes the picture characteristic of normal adult life. This is well illustrated in case two, in which after a lapse of six days after birth the blood resembled in all particulars that of normal adults, the percentage of granular cells having fallen from four to nine-tenths, and the cells still containing granules showing them diffusely scattered through the hemoglobin-containing protoplasm, cells containing granules massed in the central portion being found only after diligent search throughout several fields. The same change was noticed in the blood of the premature infant after four days in the incubator, although not to so marked an extent. The granular cells were much less numerous, and the central arrangement was not nearly so well marked.

Beside the above observations, examination of the blood to ascertain the proportion of granular cells present, and their character, was made in the following instances:

Case.	No. red cells.	Per cent Hb.	Per cent of all cells showing granules.
Pneumonia with jaundice . .	4,500,000	80	1.1
Chorea	4,300,000	80	2.6
Malaria	4,800,000	85	1.6
Typhoid fever with chlorosis	3,700,000	40	1.6
Septic anemia	2,650,000	35	1.1
	2,000,000	31	1.0
	1,000,000	12	1.1
Chorea, endocarditis, and rheumatism	4,500,000	85	1.3
Malaria	4,800,000	90	1.3

Case.	No. red cells.	Per cent Hb.	Per cent of all cells showing granules.
Convalescence from pneumonia	6,500,000	55	1.2
New growth of liver; jaundice of long duration	5,200,000	90	.8
Anemia with enlarged spleen	2,600,000	48	11.1*
Chronic alcoholism, painter for 17 years, but not at trade recently6
Rheumatism and endocarditis	3,800,000	70	.7
Rheumatism and endocarditis	3,700,000	50	1.1
Arthritis deformans . . .	4,800,000	65	.8
Carcinoma of stomach . .	2,018,000	16	1.
Gonorrhœal rheumatism, painter 18 years, but not at trade recently	4,800,000	80	.5
Secondary anemia (hemorrhoids)	3,816,000	32	.7
Aortic insufficiency . . .	4,500,000	70	0.4
Myocarditis	4,800,000	80	.5
Cerebral tumor7
Uncinariasis	3,500,000	65	1.4
Purpura simplex			1.2
Henroch's purpura8
Pneumonia	5,200,000	80	.7
Pneumonia	4,800,000	85	1.2
Pneumonia	5,300,000	85	1.
Aortic insufficiency	5,000,000	90	.7

From a study of the above table we find that the percentage of granular cells found is practically the same as that in the blood of normal individuals, with the exception of the case of anemia with enlarged spleen, in which we find the

* In this case there were 15 nucleated reds found in counting 500 leucocytes.

high percentage of eleven and one-tenth, accompanied by the presence of nucleated reds in the circulating blood. In this case cells showing all stages of karyorrhexis could be plainly made out. The case of septic anemia is particularly instructive. The low percentage of granular cells indicated an absence of any attempt at regeneration, a fact which is further established by the rapid decrease in the blood count.

Specimens of bone marrow obtained from autopsy cases were also examined by allowing the unfixed smear to come into direct contact with a drop of the stain. In these cases the percentage of granular cells seems to be about three, a larger per cent than is found in the normal circulating blood, and about the same as that obtained in the blood of newly born infants. Since, however, there are many particles of granular debris in these cases, some of which it is exceedingly hard to differentiate from granules within the cells, no stress can be laid upon the results. In addition, an examination was also made of the blood of pig embryos in various stages of development. In the latter case the blood was obtained by amputating the head of the embryo and staining the drop thus collected in the usual manner. The results obtained were as follows: The number of granular cells in a pig embryo whose length was three and one-half inches was seventeen per cent of the total number of cells seen. Nucleated red cells were present showing a blue-staining, almost homogeneous nucleus, around and in which were scattered numerous fine, pinkish-staining granules, presenting a picture such as that seen in Figure 3. In these granular cells the arrangement of the granules was for the greater part in the central portion, and many of them showed in addition numerous delicate, reddish-staining nucleoli. The cells showing granules diffusely scattered throughout were present in far less proportion than those showing the central arrangement.

In the blood of an embryo whose length was two and one-fourth inches the granules were present in twenty-four per cent of all red cells. In smears made from the liver of this embryo, and stained with polychrome, the nucleated reds

showed in almost every instance a distinct nuclear network and a well-marked nuclear membrane. It is worthy of note that in no cell of this description were granules to be found. They were, however, almost constant in those cells in which the nuclear membrane had become indistinct, and the nuclear network staining a decided purple had been replaced by a more homogeneous or finely granular mass staining blue. In the blood of an embryo seven-eighths of an inch long fully one-half of all cells seen were nucleated, and of a larger and more oval shape, probably corresponding to the ancestral cells, as described by Howell. Many of these showed granules in their hemoglobin-containing protoplasm, and practically all of the unnucleated cells seen were of the granular variety.

On staining a drop of the blood of a goose in the usual manner the following picture was obtained: Many cells were found in which the nucleus appeared to be in a perfectly normal condition. The nuclear membrane in these cases was very distinct, and the chromatin network stood out sharply defined, the whole staining a deep purple, thus showing that the structure of which it was composed contained elements which showed an affinity both for the chromatin staining principle, the methylene azure, and for the methylene blue as well. In cells with a nucleus of this description, granules were never seen in the hemoglobin containing protoplasm. Again, there were present certain cells in which both the outline of the nuclear network and that of the membrane were decidedly less distinct, although the entire nucleus still took a purple stain. In cells of this description there were always found, usually in the immediate neighborhood of the nucleus, more rarely in remoter parts of the cell, granules staining a decided pink, showing an affinity solely for the methylene azure. As the nuclear membrane and network became more indistinct, they showed less and less affinity for methylene azure, staining decidedly more bluish, while the number of pinkish staining granules in the protoplasm of the cell showed a proportionate increase. It is hardly to be doubted that this represented a

phenomenon in the degeneration of the nucleus, in which, as the nucleus died, the chromatin was cast off into the protoplasm in the form of granules, until ultimately there was nothing remaining at the original site of the nucleus except a faintly bluish staining mass of homogeneous appearance and irregular outline and entirely devoid of chromatin. In many instances the chromatin seemed to be gathered together as it was cast off from the nucleus into comparatively large, round, deeply staining nucleoli, the appearance resembling in all respects that seen in the blood of infants and in the cases of pernicious anemia after a blood crisis, as seen in Figures 5 *h* and 2 *e*. The changes seen in pigeon's blood are identical in all respects with those seen in the blood of the goose, with the exception that the granules are as a rule much finer in structure and the chromatin containing nucleoli much smaller.

Since one of the chief points raised in regard to the nuclear character of basophilic granules in red cells has been the fact that they show no affinity for methyl green, the results of an attempt to stain these granules, which are beyond cavil nuclear in origin, may not be without interest. On attempting to stain the fresh blood of the pigeon with methyl green, even when used in concentrated solution, it was found that cells corresponding to those in the first four stages represented in Figure 1 showed a nucleus staining well, but without apparent granules in the protoplasm. Many cells showed no affinity for the stain at all, appearing to entirely lack nuclei, and yet there is little doubt that they represented stages such as are depicted in the last two figures of the same plate. That some of the nuclei which did stain with methyl green had begun to cast off chromatin granules can be inferred from the fact that they stained much less intensely, and that the structure of the nucleus was not brought out with as much distinctness as in cells with well preserved nuclei. We are, therefore, I think, justified in classifying methyl green as a weak nuclear stain, and as such it is of no value in following changes which represent a disintegration of the nucleus.

When we turn our attention to the examination of fixed specimens, we at once find that the cells containing granules have for the greater part disappeared. The changes occurring in the cells as found in these specimens are, however, very instructive as compared with those previously obtained through the examination of the fresh blood. Specimens of pigeon's blood were stained with Jenner's, Leischmann's, and Wright's stain, which are the ones usually used for the differentiation of the basophilic granules occurring in the red cells. The most satisfactory results were on the whole obtained by Leischmann's stain, after the use of which the following picture was obtained: The nuclei of the various cells were stained with varying degrees of intensity. In a few specimens basophilic granules could be seen on careful examination in the hemoglobin containing portion of the cells. The majority of the cells in which the nuclei stained poorly, however, showed distinct evidence of polychromatophilia, the entire protoplasm showing a more or less marked affinity for the basic stain. This polychromatophilia seen in cells, the nuclei of which have evidently begun to undergo degeneration, strongly suggests the theory that the reason why the chromatin granules seen in the fresh blood are not present in fixed specimens is due to the fact that through fixation methods these granules are broken up and diffused uniformly throughout the cell. It may be mentioned here that one of the arguments against the nuclear origin of the basophilic granules is the fact that they are often found in nucleated cells, the nucleus of which is to all appearances intact. This may well be the case, if they simply represent the chromatin granules which have just begun to be cast off from the disintegrating nucleus.

There remains still one element of the blood to be considered, whose morphology is beautifully brought out by the use of polychrome methylene blue. In specimens of blood prepared in the previously explained manner the blood plates are brought out with great distinctness, and changes which occur in them can be easily noted.

The blood plates were first described by Bizzozero, who

considered them as a normal constituent of the blood, inasmuch as he was able to see them flowing through the vessels. Hayem thought that they represented an early stage of the red blood cell, and accordingly spoke of them under the name of hematoblasts. Many observers besides Bizzozero, among them Osler, Mondino, and Sala, and more recently Deetjen, who has been able to demonstrate ameboid movement, consider them as simple elements, while still others think that they represent the free nuclei of the white blood cells. Among the latter are Halva, Gibson, and Howell. Löwit claimed that they were not a preëxisting element, but simply a product of the white blood cell or a precipitate of the blood. Still other authors regard them as derivatives of the red blood cell; among whom are Klebs, Wlassow, Engel, Arnold, and Maximow. More recently Hirschfeld has arrived at the same conclusion, and has been able to find the "anlage" of the blood plates within the red cell, which he designates as an "intraglobular" in contradistinction to the "extraglobular" or free blood plates. He states that he was unable to differentiate a nucleus and protoplasm as occurring in the platelets. During the past year Schwalbe and Solley have also considered their source to be the red blood cell, and speak of both hemoglobin containing and hemoglobin free plates. Lilienfeld has by chemical analysis shown that the blood plates contain nuclein.

On examining fresh blood stained with polychrome, the blood plates are at first seen as unstained, homogeneous, usually round bodies, varying in size from two to five microns. Within a short time they become more apparent as they begin to take the pinkish stain characteristic of the methylene azure. At this time they also begin to increase in size, sometimes swelling to fully twice their previous dimensions, and at the same time bluish staining granules make their appearance in the previously homogeneous plate. The arrangement of these granules is most diverse, at times being scattered throughout the cell, at others being grouped together in a mass occupying the central portion, in the latter case bearing a striking

resemblance to a nucleus (Fig. 6). At this stage there always appears to be a distinct membrane surrounding the blood plate, its outline being if anything more regular than that seen in what we might term the pre-granular stage, before the stain has acted upon it. The cohesiveness of the plates at this time is very pronounced, they either adhering to the surface of the coverglass or that of the slide, and remaining motionless or nearly so, while the erythrocytes may be seen crossing the field in the direction of the current set up by moving the coverslip. Thus we find that the number of blood plates seen in a given field is practically constant, whereas that of the red cells may be constantly changing, a factor which must be taken into consideration in the ordinary method of counting the blood plates by ascertaining their proportion to the number of red cells seen in a field. After the lapse of fifteen or twenty minutes we will find that the blood plates have lost their power of cohesion, are stained a decidedly bluish color, have become very irregular in outline, and in all cases possess a hyaline, colorless, ring-like body of regular outline, which is apparently attached to them, the latter body corresponding undoubtedly to what Puchberger has recently termed the "hyalomere" as distinguished from the irregular staining portion which he calls the "chromomere." This appearance is not confined to a few of the plates, but is characteristic of all. Sometimes one obtains a picture such as that seen in Figure 6, as if two hyalomes were attached to a single chromomere on opposite sides, but this is the exception and not the rule, there usually being only one of the former to each of the latter. After this change has taken place the blood plates apparently remain in the same state for an indefinite period of time.

Does the behavior of the blood plate toward polychrome methylene blue furnish us with any evidence concerning its structure or the source of its origin? It is not the intention of this paper to enter into any detailed discussion with regard to this subject. The fact, however, that they stain with methylene azure and behave toward dilute acetic acid and hemolytic agents as do the nuclei of red blood cells, seems to

be strong proof in favor of their nuclear character. Whether they represent individual elements or arise from the nuclei of the white cells or those of the reds one cannot say. It is interesting to note, however, that they apparently bear a rough numerical relation to the number of cells showing granules, being proportionately more when these are less and less when the granular cells are largely in evidence. Thus in all cases of pernicious anemia which were examined the number of blood plates seen was remarkably small, while that of the granular cells was, as before mentioned, greatly increased. In fact, one might say that the small number of platelets found in these cases is as striking a characteristic of the blood picture as is the large proportion of granular cells seen. In secondary anemias, on the other hand, in which no evidence of regeneration was seen, as indicated by the absence of an increase in the number of granular cells as well as a constantly falling blood count, the number of platelets was not found to fall below that seen in the case of normal blood and in various diseased conditions in which the blood picture is not particularly altered. Indeed, in two cases of marked secondary anemia they were decidedly increased in number.

To return to the granules occurring in the erythrocyte, what conclusions may we be justified in forming with regard to their nature and clinical significance? Do they represent simply artifacts, or inherent changes within the red cell itself, either in its stroma, hemoglobin content, or preëxisting nucleus, and if so are these changes of a degenerative character? It is evident that these questions must be answered before any logical attempt can be made to understand their clinical significance.

That they do not represent artifacts is well brought out by the fact before mentioned, that they do not increase in number on allowing the specimen to stand, and that the percentage of such cells seen in the blood of normal individuals, and in that of those suffering from diseases in which the blood picture is not particularly affected, is singularly

constant. That their appearance is not due to alteration in the hemoglobin content of the cell can be judged by the fact that in the cells in which they are most abundant the hemoglobin is of normal appearance and distribution. Are they, then, remnants of a nucleus which has disappeared either through a process of karyorhexis or karyolysis? All of the experimental evidence obtained certainly points conclusively in this direction. For example, they are found in greatest abundance in those cases in which nucleated red cells are also seen in the circulation, namely, in the blood of pernicious anemia and in that of newly born infants. Moreover, the fact that in these cases they occupy more nearly the central portion of the cell, the original site of the nucleus, and are at the same time of larger size than those found in the normal circulating blood of healthy adults, is a point in favor of their nuclear origin. Again, the fact that they are found in small numbers in normal blood uniformly scattered throughout the cell, and invariably of the smaller size, cannot be taken as evidence of their being non-nuclear in origin, since it is certainly reasonable to suppose that if there is in truth an intracellular disappearance of the nucleus, cells containing nuclear remains should be found in the circulating blood. That no red cell, but a fully developed normal erythrocyte, is allowed to escape from the bone marrow, even in conditions of health, is not at all likely. Furthermore, these granules show a pronounced resemblance to those described above, as seen in the blood of the pigeon and goose, and which were undoubtedly composed of chromatin derived from the disintegration of the nucleus. In addition, they show a decided affinity for methylene azure, which is conceded to be a specific chromatin stain.

Regarding the manner in which the nucleus undergoes disintegration within the red cell, the above researches furnish the following information:

The well-preserved nucleus of the erythrocyte shows a distinct nuclear network and a sharply defined nuclear membrane, the whole staining a decided purple with polychrome methylene blue. Such cells show usually no granules in

their protoplasm, or at the most one or two small ones situated in the immediate vicinity of the nucleus. As degenerative changes advance, however, the nuclear network and membrane become more indistinct, the nucleus becoming more irregular in outline, and the chromatin being gathered together in the form of round nucleoli of various sizes, staining red with methylene azure. These nucleoli are cast out into the surrounding hemoglobin containing protoplasm, where they become more and more broken up into the finest granules and ultimately disappear. For a short time after the gathering together of the chromatin an irregular staining mass may be noticed at the original site of the nucleus, which quickly disappears altogether.

Considering the above mentioned granules, therefore, as of nuclear origin, what significance can we attach to them either from a diagnostic or prognostic standpoint? It is evident that their importance depends solely upon whether they are present in a large proportion of all cells or not. When they are present in a larger percentage than normal among the cells of the circulating blood, the same value can be attached to them within certain limits as can be given to the finding of nucleated red corpuscles. The increase in the number of such cells may, however, be noted, long before the discovery of nucleated cells themselves, and thus may lead to the suspicion of the presence of certain conditions at an early period. An increase of the granular cells to nine or ten per cent of the total number of cells seen would indicate a marked departure from the normal and would probably not occur in the absence of nucleated forms, although the latter might be found only after diligent search. As regards prognosis, their significance would depend entirely upon the condition in which they occurred. For example, in cases of secondary anemia a high percentage of granular cells would be looked upon as a favorable sign, indicating a state of blood regeneration, whereas in the case of pernicious anemia, its value as a prognostic sign would be practically nil—the rise of the blood count and the hemoglobin index being much more favorable signs in the latter case.

The simplicity of the technic and the fact that by its employment not only granular cells, but both nucleated reds and differentially stained leucocytes, as well as the malarial parasite, are well brought out, would seem to warrant the assumption that the method may be of some little aid to clinical hematology.

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A STUDY OF VOLUME INDEX. OBSERVATIONS UPON THE VOLUME OF ERYTHROCYTES IN VARIOUS DISEASE CONDITIONS.¹

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Influences affecting the size of erythrocytes. — The size of the erythrocyte depends upon two essentially different factors: chemico-physiologic and what may be termed biotic.

(a.) The chemico-physiologic influences relate chiefly to the process of osmosis. That the red corpuscle may change its size under the action of various fluids is a matter of common observation. Thus a fluid of the same molecular concentration as the blood serum (isotonic) does not affect the red cells. A concentrated solution of salt (hyperisotonic) causes the corpuscle to take up the salt and give up its water, thereby producing the microscopic picture of crenation and shrivelling. A solution of much less molecular concentration than the blood serum, *e.g.*, water (hypoisotonic), forces the cell to give up its hemoglobin and to absorb water. Hence we see the red corpuscle swollen and pale from the loss of its pigment.

(b.) The biotic influences may be described as anabiotic and katabiotic. Anabiotic changes are those concerned with the growth and development of the cell.

In the light of Pappenheim's researches on the bone marrow, we must consider that the prototype of the erythrocyte is a mononuclear cell, which gradually develops into the megaloblast. From the megaloblast is evolved the normoblast, which extrudes its nucleus and becomes a normal red corpuscle. A macrocyte may be produced from the megaloblast under certain conditions in the same way that the normocyte is from the normoblast. Thus it is seen that the red corpuscle varies in size at different periods of its life history. In favor of large cells being youngest is their

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prevalence in the bone marrow of embryos where both megaloblasts and macrocytes preponderate, while after birth only the normocytes are found. Moreover, the mitoses are nearly always seen in the megaloblasts. In exceptional conditions red cells may be generated not by mitoses, but by direct cell division. In this event the young cells are small (Schistocytien of Ehrlich).

Katabiotic changes are those that have to do with cell degeneration. So far as we know, the degenerating cell tends to become smaller and deformed (poikilocytosis).

A cell that is Hb poor as a result of osmosis should be swollen and increased in volume. Hence a cell that is pale but small does not owe its Hb impoverishment to osmosis, but to some nutritional or degenerative disturbance. Likewise a macrocyte rich in Hb cannot result from simple osmosis, but must be the result of abnormal cell development.

Both unripe macrocytes and the degenerated microcytes are polychromatophilic, while the cells influenced by osmosis alone do not show this tendency.

Poikilocytosis is an expression of biotic degeneration or of regeneration, not of osmotic changes.

Cell size, therefore, is not in itself a reliable index of its age. Moreover, we cannot say from the size alone whether a large or small cell owes its volume to metamorphosis or osmosis or both together. Other associated conditions of the cell must be studied, such as its staining properties, its shape, and its hemoglobin content. The last-named condition of the erythrocyte has been exhaustively investigated throughout the range of disease, but not in its close relationship to the cell volume. This neglect is chiefly attributable to imperfect methods of determining the size of the cells.

Prevalent methods of estimating the size of the erythrocytes. — The usual method is to measure the cell diameter with a micrometer scale which gives only approximate results, even in normal blood. In this way only one dimension

of the cell is obtained. The erythrocyte is a bi-concave disc, and may approach a spheroid form without increasing the diameter laterally. When the cell is caudate or elliptical in contour all measurements are fallacious. The only way to ascertain microscopically the thickness of the cell is by observing it on edge or by noting the presence or absence of a central light area. A method which gives the cell dimensions in but one direction, especially in diseased blood where poikilocytosis is the rule, is obviously inaccurate and misleading.

Methods for estimating the volume of erythrocytes.—M. and L. Bleibtreu¹ have introduced a chemical process for this purpose. They mix the blood with physiological salt solution and after it has settled estimate the amount of nitrogen in the supernatant serum. According to a mathematical formula, the volume of serum and cells is computed from this nitrogen content. The method requires a large amount of blood, is complicated, and is open to criticism for inaccuracy.

Spontaneous sedimentation is advocated by Biernacki,² who obtains several cubic centimeters of blood by venesection, which is mixed in a graduated tube with oxalic acid and sodium oxalate solution to prevent coagulation and allowed to stand twenty-four hours. The column of precipitated corpuscles is then measured. Biernacki considers the speed of sedimentation, as well as the sediment volume, of clinical importance. Grawitz³ has devised a blood voluminometer based on the principle of spontaneous sedimentation and endeavors to make the test possible with small quantities of blood. He covers the inside of a graduated tube with sodium oxalate powder and measures the relative spaces occupied by serum and cells.

The hematocrit was first used to estimate cell volume by Blex-Heidin⁴ and later by Gärtner⁵ and Daland. A small

¹ Pfüger's Archiv., Vol. li, 1892.

² Zeitschrift f. klin. med., Vol. xxiv, 1894.

³ Grawitz. Pathologie des Blutes, Berlin, 1902.
Skandinav. Archiv. f. Patholog., 1890.

⁴ Berlin klin. Wochens., No. 36, 1892.

quantity of blood is sucked up into a capillary tube, diluted with a two and one-half per cent bichromate of potash or normal salt solution, centrifugalized, and the cell mass measured on a scale.

The centrifuge is not only speedier than spontaneous sedimentation, but produces a much sharper separation of serum and cells.

But all methods are unsafe which require the mixing of blood with salt solution in order to avoid coagulation. A normal salt solution will affect the cell size of one blood and will not that of another. For every individual blood a special test must be made, in order that the solution may be isotonic. Obviously such a preliminary test is impracticable for clinical purposes.

Herz¹ has endeavored to dispense with salt mixtures by rendering the walls of the pipette smooth by the application of cod liver oil. Koeppé² accomplishes the same purpose by the use of cedar oil. The oil is entirely separated from the blood and collects at the inner end of the tube. The relation of the layers of plasma and corpuscles is read off in the usual way on the graduated tube.

I have used the oil method frequently and believe it is a long step in advance of the employment of salt mixtures. But even oils may affect cell volume and are objectionable. The best results, in my opinion, are obtained by the use of blood only. Two conditions are essential to prevent coagulation, viz., scrupulous cleanliness of the tubes and speed in operation. The latter condition requires that the blood must be placed in the hematocrit within a few seconds of withdrawal. In office practice I use the electric centrifuge, but in the hospital or in private I employ the hand hematocrit, which can be set up in the vicinity of the patient and is equally reliable. It is desirable always to fill two tubes as a control of one's results. The machine should be operated for three minutes at a uniform speed of ten thousand revolutions a minute.

¹ Virchow's Archiv., Vol. cxxxiil.

² Archiv. f. Anat. v. Phys. Abstract, 1895.

A new method for determining the average volume of the individual erythrocyte. — This method requires the use of the centrifuge in conjunction with a careful count of the red cells with a Thoma-Zeiss instrument. The blood is allowed to run into the graduated tube of the hematocrit, which is quickly centrifuged and the height of the column of red corpuscles read off. The percentage volume of the red cells is then divided by the percentage number of cells (as obtained by the Thoma-Zeiss apparatus), and this gives the percentage volume of the individual corpuscle. This quotient I have previously named Volume Index,¹ which is easily associated with the familiar term Color Index. Let us suppose, for example, the red cell column in the capillary tube reaches forty-five or ninety per cent, the red cells number 3,750,000 or seventy-five per cent; then $90/75 = 1.20$, the volume index, representing an increase of twenty per cent above the normal in the volume of the average cell.

In the following observations the volume index was ascertained along with the color index. For the sake of comparison I have frequently measured the cell diameters, in all instances taking the average of one hundred cells.

Observations upon normal blood. — (Table I.) The following examinations of ten healthy individuals afford an idea of the working error of the method and of the differences found in normal blood. The variation in both color and volume index is about ten per cent. The average cell diameter is 7.65 microns. These normal fluctuations are given due weight in the subsequent study of diseased blood.

The average hematocrit reading is forty-eight, being forty-six and one-half for females and fifty and one-half for males. These readings correspond closely to recent observations of Grawitz, who found averages respectively of forty-five and fifty.

In estimating the color index the Fleischl reading is always corrected by adding ten per cent. Thus a reading of seventy is corrected to seventy-seven per cent.

Pernicious anemia. — (Table II.) The thirty cases herewith reported are typical of the disease, showing an average of

¹ Journ. Am. Med. Ass'n, Feb. 16, 1901.

thirty-two per cent hemoglobin and 1,458,000 erythrocytes, which give an average reading of 19.4 on the hematocrit scale. The average color index is 1.27, the volume index 1.37, or ten per cent higher than the former. The diameter of the red cells is likewise increased and averages 8.03 microns.

Hyperchromemia is one of the essential characteristics of pernicious anemia, for the explanation of which many reasons have been advanced. The two most plausible theories are the following:

(a.) An increased affinity of the cell protoplasm for hemoglobin.

(b.) The presence of many macrocytes which by their richness in Hb raise the color value.

The first theory of heightened affinity is upheld by Von Jaksch,¹ who found that one hundred grams of fresh red corpuscles in pernicious anemia contained 6.48 grams of nitrogen instead of the normal 5.5 grams. From this experiment he concluded that every unit of protoplasm contained a hyper-normal proportion of albuminous elements, *i.e.*, hemoglobin. According to this theory, a high color index might be present, although the cells were normal or diminished in size.

Our observations directly oppose this hypothesis. The color index never is increased without a corresponding or greater rise in the volume index. This fact proven, it is quite unnecessary to assume a supersaturation of the cell with Hb.

The second theory has much to commend it, even when the centrifuge is not employed, for macrocytes, as a rule, are numerous in pernicious anemia. It is contended, however, that the microcytes neutralize this gain in cell protoplasm. Hitherto the whole matter has been largely one of speculation, owing to the unreliability of the prevalent method of measurement. The many oval, caudate, and other irregularly shaped cells characteristic of pernicious anemia present special obstacles to accurate measurement. The spherical form of the Eichorst's cells is also a prolific source of error.

¹ Ebstein and Schwalbe.

A comparison of the diameters with the volume indices in the accompanying table illustrates the fallacies of measurements. Thus Case 3, with a volume index of 1.39, gives an average diameter of only 7.54 microns, which is less than normal. This was due to the presence of many Eichorst's cells, which present no increase in diameter, but contain far more protoplasm than the normal red cell.

Case 6, with a cell diameter of 8.32, has a volume index of 1.21, whereas Case 1, with a diameter of 8.26, has a volume index of 2.00.

The more pronounced the variation in cell size as well as in cell shape, the more untrustworthy are the average diameter measurements. On the other hand, the accuracy of the centrifugal method of estimating cell volume is not affected by variations in either size or shape of the cell.

Relation of volume and color indices. — It is generally true that the volume and color indices in pernicious anemia are highest when the red count and Hb value are lowest, but this relationship is not at all constant.

The diagnostic value of the high color index is well established. There are often cases in the milder stages of the disease which present a normal or subnormal color index. In such instances the volume index is very valuable, for the volume index is always as high and usually higher than the color index. This difference amounted on the average to ten per cent. The volume index is more constantly above normal and consequently more reliable as a sign of pernicious anemia than the color index.

The cases in Table III. illustrate the behavior of the blood during improvement and decline. The volume and color indices steadily decrease with the betterment of symptoms and rise with the downward progress of the disease.

This suggests what is usually true, that the prognosis is worse when the volume and color indices are high.

Secondary anemia. — For the sake of convenience I have divided a number of symptomatic anemias due to miscellaneous causes into three groups:

- I. Anemias of moderate degree.
- II. Severe acute anemias.
- III. Severe chronic anemias. (Tables IV., V., VI.)

The first group is made up of patients who for the most part were ambulatory. The great majority give evidence of a moderate loss of cells and a somewhat greater loss of hemoglobin. The hematocrit reading falls only a little below the normal and gives an average volume index of .95. There are a few notable exceptions where the cell volume has been a greater sufferer, *e.g.*, in the gastric cancer, salpingitis, and the two cases of rhachitis. In most of the moderate grades of secondary anemia, although the color index falls considerably, the volume index is not greatly decreased.

The second group of severe acute anemias illustrates the effect of virulent hemolytic toxins on the blood. All of these cases terminated fatally. The great reduction of red cells and hemoglobin, which average respectively 2,573,000 and twenty-eight per cent, is noteworthy. Compared with Group I. both color and volume index have suffered severely. Especially is the cell size attacked. In ordinary secondary anemias the Hb loss is from two to ten times greater than the loss in size. In these septic cases, however, the volume loss almost equals the Hb loss. The volume index of .59, observed in Case 4, is the lowest I have seen in any condition.

Microscopically the field is pervaded with microcytes and poikilocytes. Macrocytes are conspicuously absent. In none of the smears was a cell found over ten microns in diameter. The cells appear thin and poorly colored. Poikilocytes are so numerous that measurements of the cell diameter is almost valueless.

The acute septic anemias produce to an exquisite degree the microcytic blood which may be looked upon as the result of direct degenerative changes.

The "acute swelling of the red corpuscles without a rise in hemoglobin," which is described by Herz as characteristic

of acute anemias, I have never observed. Indeed, fewer large cells are seen in these septic cases than in any other diseases.

Group III. includes only high grade anemias of a chronic type. The average red count of 2,220,000 is even less than that seen in the last group. Yet in other respects the blood findings afford a remarkable contrast. The color index of .80 represents only a moderate decrease, and the volume index of .95 is not far below the normal. In four cases the volume is actually above 1.00. Macrocytes varying in size from ten to thirteen microns were present in all but three cases. Poikilocytosis was always present, but seldom pronounced.

Some of these long-standing secondary anemias closely resemble the blood of pernicious anemia, exhibiting a high volume and a relatively high color index. Nor does the similarity cease here. Cases 10 and 11 (uncinariasis and splenic anemia) show both megaloblasts and normoblasts, as well as marked poikilocytosis and polychromatophilia. The blood of these cases is almost identical with that of pernicious anemia. Earlier examinations of the same patients showed the chlorotic type with lowered color and volume index. This transition in secondary anemia from the microcytic to the macrocytic blood has been often recorded. The hook worm usually causes a chloranemia with undersized erythrocytes, but some cases are recorded (Neusser, Grawitz) where the red cells in the advanced stage of the disease, as in our case, became larger and showed a rise in color and volume index, presenting the characteristics of pernicious anemia. Most cases of severe chronic symptomatic anemia, however, do not show this marked tendency to macrocytic blood. They present to the last the microcytic, atrophic type.

The following generalizations may be made:

In secondary anemia with a moderate oligocythemia, the erythrocytes, as a rule, sustain a slight loss in size, but when the anemia is severe we usually find the cells much undersized and atrophic.

A certain number of the chronic anemias, best illustrated

by splenic anemia and uncinariasis, exhibit in the earlier and milder stages a typical chloranemia with low color and volume index; at a later stage characterized by a very low count of red corpuscles, the red cells may become larger, of the macrocytic type, with a high volume index, a type that closely resembles pernicious anemia.

The acute severe anemias resulting from septicemia are characterized by the microcytic type of blood with an exceedingly low volume index.

Chlorosis. — It has been generally held that the erythrocytes in chlorosis are smaller than normal, but how much smaller and under what conditions they are dwarfed has never been accurately determined.

Laache¹ found the cells in chlorosis varied from four and four-tenths to nine microns; Schauman and Wellebrandt² from four and two-tenths to nine and eight-tenths microns. In harmony with these observations are those of Strauss and Rohnstein,³ and Van Noorden,⁴ although in exceptional cases they found macrocytes over ten microns in diameter.

On the other hand, an "acute swelling" of the cells is described by Herz. Lazarus⁵ says that measurements do not bear out this assumption, but that the cells are probably increased in thickness. Herz cites an instance of hematemesis where the cells averaged three times the normal size.

Malassez⁶ thinks that in spite of the presence of many small cells in chlorosis, there are enough large cells to bring the average above the normal.

From these citations we see that even among authorities there is no unanimity of opinion in regard to the size of the chlorotic cell.

The following observations demonstrate that the determination of cell volume is a valuable aid in the understanding of

¹ Die Anæmie, Christiana, 1883.

² Congr. f. innere Med., No. 22, 1896.

³ Die Blutzusammensetzung bei d. Verschied. Anemia.

⁴ Nothnagels spec. Pathol., Bd. viii, p. 36, 1901.

⁵ Die Anæmie, Wien, 1898.

⁶ Archiv. de Physiol., p. 652, 1887.

chlorosis. The cases of chlorosis may be advantageously studied under two groups :

I. Those having a hemoglobin percentage of more than fifty (Table VII.).

II. Those having a hemoglobin percentage of less than fifty (Table VIII.).

Such a classification roughly separates the milder from the more severe cases.

The average number of red cells in the twelve cases of the first group is 4,450,000, which is practically normal. The oligochromemia characteristic of chlorosis is indicated by the average hemoglobin of .63 and the color index of .78. The volume index is within normal limits in only four of the cases and averages .92.

Comparing these averages with the table of mild secondary anemias, one sees qualitative differences in the blood changes. The red count is nearly the same in each group, and yet Hb per cent and color index have both fallen ten per cent lower in the chlorosis. Equal in significance is the fact that the volume index has fallen lower in mild chlorosis than in the mild secondary anemias.

In the nine cases of Group II. there is a general loss in blood values, color, volume, and number of reds. The cell volume has suffered in every instance and usually to a marked degree. Let us refer for comparison to the group of severe secondary anemias, where the average red count was 2,440,000 as against 3,692,000 in the chlorotic group.

In the secondary anemias the average color index is .80, while in chlorosis it is .54, illustrating the intense poverty of the chlorotic cell in coloring matter. The average volume index in the secondary anemias was .95, that of chlorosis .72, which shows the greater atrophy of the chlorotic cell. Microscopically the erythrocytes are undersized, pale, and have large areas in the center. Microcytes abound and give the picture of "atrophic" cells. In the worst cases (*e.g.*, Case 4) poikilocytosis is present. Macrocytes are usually not observed, at least of the larger variety, but cells measuring

ten microns are not uncommon and a few were seen eleven microns in diameter (Cases 3 and 4).

Of the twenty-one cases in both groups of chlorosis, only four are seen to have a normal volume index. These four exhibit very little diminution in the red count and the Hb is in no instance below sixty. Clinically they represent the very mildest grade of chlorosis, and they all responded well to treatment (1-3-6-12). We cannot assume, however, that every case with more than sixty per cent Hb has a normal volume index, for three cases with readings of 70, 72, and 70 respectively, exhibit a reduced volume index. And these latter cases did not respond as well to treatment as the former.

Is the number of red cells in chlorosis an indication of cell volume? A blood with a normal count may have a volume index lower than one in which the count is decreased. For example, in Case 1, Table VIII., with a normal count of 4,620,000 the volume index is .79, while in Case 5, Group I., with a count of 3,832,000 the volume index is .85. The very lowest volume index of the series is .62, which occurs with 4,160,000 erythrocytes. Other instances abound to impress us with the belief that the cell count cannot be depended upon to enlighten us concerning the size and volume of the cells. It is quite safe to say with a count of less than 4,000,000 that the cells always suffer some loss in volume. On the other hand, when the count is normal or over 4,000,000, the cells may be either full sized or they may show an extreme degree of atrophy.

Can any relationship be established between the volume of the cells and the Hb content? Were a chart made to compare the Hb percentages with the corresponding volume indices it would be observed that when the Hb is above sixty per cent, the volume index may be normal or may be decreased; when the Hb is below sixty per cent, the volume index is invariably diminished. So it may be said with probability that every case of chlorosis with a Hb per cent of less than sixty presents a distinct pathological decrease in the size of the erythrocytes, and this holds true whether the

number of cells is diminished or not. The volume index bears a more constant ratio to the Hb percentage than to the number of cells.

To what extent this atrophy takes place in a given case cannot be ascertained without the use of the centrifuge. A knowledge of cell volume is one of the best means of determining the bone marrow activity, for the production of dwarfed erythrocytes is an evidence of morbid bone marrow function quite as much as the production of too few cells.

The discussion naturally brings us to the question of the bearing of cell volume upon prognosis and treatment. Does the dwarfed cell take up iron as readily as the full sized cell? Is recovery slower in the cases where atrophy is pronounced? The following cases were examined repeatedly during treatment to determine these points (Table IX.):

Case I. is a type of mild chlorosis. At the first examination the red count was normal and the volume index was not diminished. Under treatment the Hb, and with it the color index, steadily advanced, until at the end of three months the blood was normal and the clinical symptoms had disappeared. The process was simply a rapid acquirement on the part of the cells of the deficient hemoglobin. The microscopic appearance of the erythrocytes was not abnormal in any way, except for the pallor which disappeared with treatment.

The instructive feature of Case II. is the effect upon the blood of a remission in chlorosis due to neglect of treatment. The first month of treatment succeeded in increasing the Hb by ten per cent and, what is more important, in restoring the volume index to normal. During the following month all treatment was suspended and the old symptoms recurred. A Fleischl reading recorded only a slight loss (2 per cent) of Hb, but the volume index had sunken very materially. Upon renewal of treatment both volume and color responded and later the cell count. The patient was practically well in three months.

The pronounced shrinkage of the cell volume during the remission calls for explanation. We know that the Hb

content is quite rapidly and easily disturbed. In ordinary anemias the volume is more resistant. A rational interpretation is that in the process of recovery the erythrocytes had not yet gained their usual stability and relapsed quickly into their dwarfed condition when no longer stimulated by treatment.

In Case III. the earliest blood count gave only a moderate diminution in the number of red cells, but a reading of thirty-six per cent Hb. The color index was .47. The centrifuge showed the astonishingly low reading of 28, which corresponds to 2,800,000 normal cells, whereas in fact the count was 4,160,000. The resulting volume index was .62, which represents an average loss of nearly forty per cent in protoplasm in the individual cell. In the coverglass preparations most of the erythrocytes were undersized and true microcytes were numerous. Many were oval shaped, but poikilocytes were exceptional. Here and there a cell measuring as high as ten microns could be seen. The average diameter was seven and twelve-hundredths microns. After two weeks of treatment with iron and arsenic the patient said that she felt much better. The second blood examination revealed practically the same Hb and red count as before. The only change found was a slight rise in specific gravity and a distinct increase in the blood column of the hematocrit, which was 2.9. Thus the volume index rose from .62 to .70, while the amount of Hb and number of cells remained stationary. Measurements of the cells confirmed this observation, the average diameter increasing from seven and twelve-hundredths microns to seven and thirty-eight-hundredths microns.

At the next examination the volume index showed a substantial increase, while the Hb gained but six per cent. Subsequently the Hb made gradual small gains month by month, always accompanied and preceded by expansion of the cell volume. The cell volume reached the normal limit four months in advance of the Hb. A full year elapsed before the patient was discharged cured, although she faithfully

kept up the treatment. Doubtless the course would have been shorter by bed treatment.

From these observations it is clear that the blood restoration included two distinct processes: (*a*) a growth of the erythrocytes, (*b*) the taking up of hemoglobin. During the first two weeks, when the Hb was stationary, the bone marrow was not by any means idle; it was manufacturing not more cells, but larger ones. The cell growth preceded the rise in hemoglobin. Now as we would naturally expect, a comparatively long period elapsed before the bone marrow produced full sized erythrocytes, and necessarily the Hb percentage during this period could not reach normal. I say necessarily, for supposing the cell has a volume index of .70, the Hb content or color index cannot exceed .70 unless the protoplasm passes the "point of saturation." All our observations bear evidence against such an occurrence. In fact, during the period of recovery the volume index keeps ahead of the color index by a wide margin.

Case V. as regards poverty of Hb and red corpuscles is by far the severest of the series. Clinically also she suffered from the most marked symptoms. Nevertheless, recovery was more rapid than in Case III., who at the beginning of treatment possessed almost twice the number of cells and twice the amount of Hb. Making all due allowances for individual differences in the patients, it seems probable that the relatively better cell volume in Case V. gave her distinct advantage in the race for recovery.

It seems easier for the bone marrow to correct a quantitative deficiency than a qualitative one. For example, the loss of a million cells from hemorrhage in a normal person is made up in short time, but the restoration of microcytic blood to normal size requires a considerable period. Upon this ground we can understand how a case of chlorosis having a low red count and Hb with a relatively good cell volume may offer a more favorable prognosis than a case having a higher red count and Hb with a relatively poor cell volume. The cell volume, therefore, is an important factor in making the prognosis in a given case of chlorosis. A low

volume index indicates a tedious recovery, even if the red count is not much decreased. We may reasonably hope by this method to obtain a clearer insight into the nature of individual cases of chlorosis.

Dropsy in nephritis. — (Table X.) In order to study the specific influence of hydremia on the erythrocyte, I have selected only extreme grades of dropsy, all of nephritic origin. Care was taken to make a deep skin incision, and the blood was withdrawn from a part showing the least edema. Cases accompanied by marked cyanosis were excluded for fear of complicating the results. As a control upon the experiment a group of nephritic cases without dropsy was also investigated.

A comparison of the two groups reveals a surprising difference in blood values in favor of the dropsical cases. These higher values affect equally the Hb, specific gravity, and red cells. Both color and volume indices are relatively well preserved in the dropsical, while they sustain considerable loss in the interstitial cases. Especial interest attaches to the volume index, which is usually normal or slightly diminished.

Herz states that dropsy from any cause brings about a "hypertrophic swelling of the red cells." The centrifuge fails to support his contention. Furthermore I am unable by microscopic examination to observe any swelling of the cells. The average diameter is not above 7.7 mikrons in any of the series. Macrocytes are rare, seldom exceeding nine microns in diameter.

The cells appear well colored and are not polychromatophilic. To find blood values in dropsical nephritis so much higher than in the interstitial forms is surprising when one considers the marked pallor presented by most of the patients with parenchymatous nephritis. Some indeed appeared almost bloodless. The pallor is probably due to vasomotor disturbance. The pressure of the fluid deprives the skin and peripheral parts of their natural blood supply. Another manifestation of the capillary circulation disturbance is the cyanosis which so often occurs with hydremia.

An analogous local pallor is seen in angio-neurotic edema or in the skin surrounding a fresh subcutaneous infusion.

To summarize, the nephritic dropsy is characterized by

(a.) A blood much richer in hemoglobin and erythrocytes than we would suspect from the appearance of the patient.

(b.) Erythrocytes well preserved or moderately shrunken. Cell hypertrophy cannot be demonstrated either by the centrifuge or by cell measurement.

Jaundice. — (Table XI.) The first group of six cases of catarrhal jaundice exhibit blood findings that are normal, allowing for a mild grade of anemia.

In the second group, comprising six cases of jaundice from gall-stones, the degree of anemia is more marked, but otherwise the erythrocytes are not characteristic.

Group III. includes deep jaundice occurring in the course of carcinoma of the liver or pancreas. These findings show a considerable degree of anemia with sixty-five per cent Hb and 3,665,000 reds. In spite of this fact the color and volume indices are higher than in Group II. Furthermore, the color index equals or exceeds the volume index in three of the six cases.

All the color tests are of little worth in jaundice because the bile pigment tends to intensify the blood color and exaggerate the Hb value. In this way Hayem's¹ high color indices in biliary cirrhosis can be explained.

The high volume index in two cases, both with marked oligocythemia, must be due to a real expansion of the cell. Measurements of the cells in Cases 13 and 18 give an average of 7.66 microns and 7.85 microns respectively, a slight increase. The enlargement apparently affects all the cells equally, for no true macrocytes are present. No cells were seen over ten microns in diameter and no poikilocytosis was observed.

This swelling of the cells first described by Limbeck² in

¹ Pathologie du Sang, Paris, 1878.

² Cent. f. innere Med., Nr. 33, 1896.

catarrhal jaundice was not in evidence in any others of the series. As a rule the microscopic appearances were indicative rather of shrinking, since rapid crenation took place in a large number.

Rouleaux formation as a rule was interfered with, but not invariably absent, as stated by Grawitz to be true.

Considering the eighteen cases of jaundice as a whole we may conclude

(1.) That the Hb estimate is unreliable and the high color index consequently of no moment.

(2.) The erythrocytes display a strong tendency to rapid crenation and little tendency to rouleaux formation.

(3.) With two exceptions the erythrocytes do not undergo the swelling described by Limbeck. The above-named exceptions were carcinomata of the liver and showed a uniform enlargement of the red cells without the presence of true macrocytes.

Cyanosis.— (Table XII.) Before considering the fourteen cases of cyanosis, attention should be called to the fact that many of them are subject to disease usually associated with anemia. In other words, some of the results represent an anemia combined with cyanosis.

Polycythemia, increased specific gravity, and the comparatively high Hb value are the noticeable features of the blood. The volume and color indices never exceed the normal. Cell measurements give similar results, and the shape of the cells is unaffected.

The dark-colored blood does not impair the test for Hb, because the mixing process converts the methemoglobin into oxyhemoglobin.

Cyanosis seems to cause a simple concentration of the corpuscular elements without changing the size of the individual erythrocytes. The rapidity with which this concentration may take place is well illustrated by Case 14—a woman with cardiac neurosis. During an attack of palpitation, with a pulse rate of one hundred and ninety, and extreme cyanosis, the blood findings were Hb ninety per cent, reds

6,400,000, hematocrit 62. Fifteen minutes later, when the palpitation suddenly ceased and cyanosis cleared up, she showed Hb eighty per cent, reds 5,550,000, hematocrit 54. The volume index in the first examination was .97, in the second .98.

Hemorrhage — Acute.— (Table XIII.) I was fortunately enabled to examine a case of gastric ulcer soon after a very copious hemorrhage, and to carry out further observations until the blood was nearly restored to the normal. The blood changes may be considered in four stages:

First a short period after the bleeding ceases, when the blood grows thinner and the cells fewer. This may be due to a continuation of capillary oozing and to a gradual thinning of the blood by absorption of fluids. This quiescent stage lasted three days, and probably was in part attributable to the depressing effects of shock on the bone marrow activity.

A second stage, in which occurs a remarkable increase in the number of erythrocytes, a large portion of which are small and poorly colored. A few erythrocytes show a tendency to hypertrophy, so that the variation in size of the cells is marked. Normoblasts are present. The hemoglobin begins to rise, but lags far behind the increase in red cells. As a result we see a considerable fall in the color index. A drop even more pronounced in the volume index takes place as a consequence of a sudden influx of erythrocytes poor in protoplasm. These microcytes are apparently newly-formed cells, and appear to be fragments chipped off the older cells.

In the third stage, a few days later, although the blood is receiving fresh recruits from the bone marrow, there are fewer microcytes, and the volume index tends to rise again. The color index, on the other hand, falls still lower because the hemoglobin is not able to keep pace with cell regeneration.

Lastly, where recovery takes place without interruption, the further enrichment in cells and hemoglobin is more nearly proportional. The normal number of erythrocytes is attained, however, long before the hemoglobin is restored.

But these new cells are undersized for a long time, as shown by the subnormal volume index. So long as the volume index is below 1, the hemoglobin must also be deficient.

Chronic hemorrhage.— (Table XIV.) Hemorrhages of a subacute or chronic nature produce various blood pictures dependent upon the amount and duration of the bleeding on the one hand, and upon the vitality and recuperative power of the bone marrow on the other. The extraction of a quart of blood would not necessarily affect two individuals the same way; and, furthermore, the reaction of the same individual might be quite different after a second hemorrhage from that following the first.

The effect of shock from a copious loss of blood, so well illustrated in Table XIII., is to depress the bone marrow function so that for a short time no reproduction takes place. A somewhat analogous condition may be seen after repeated severe hemorrhages where the regenerative process is slow and where the erythrocytes produced are undersized.

Slight bleedings, even when often repeated, may cause little alteration of the blood (see Cases 9 and 10).

As illustrations of severe subacute or chronic hemorrhages, the accompanying cases are recorded in their order of chronicity. Contrasting the first four recent cases with the last four long standing cases, we see no material difference in the number of red cells. The latter group shows a strikingly low color index and volume index. As hemorrhages become more chronic, the cells become smaller and poorer in hemoglobin.

Repeated hemorrhages of moderate severity seem not to seriously interfere with the production of full-sized erythrocytes. After the blood has been depleted, however, by successive severe hemorrhages, the bone marrow is able to produce only the dwarfed microcytic red corpuscles. A few large cells are usually seen, but they are so sparse that they serve to accentuate the general picture of atrophy.

These observations directly conflict with the assertion of

Herz¹ that an "acute swelling" of the red cells follows a severe bleeding.

Lazarus² also describes a simple swelling which he attributes on theoretical grounds to osmotic absorption of the watery serum. He assumes that the blood undergoes a loss in molecular density after hemorrhage.

More recent investigations, however, by Schreiber and Hagenburg³ show that the freezing point of the blood does not change before and after hemorrhage. The fluid which flows into the blood vessels from the lymph spaces is of the same molecular weight as the blood serum itself. Hence no osmotic swelling of the erythrocyte would be expected.

Grawitz⁴ questions the existence of such a swelling from the microscopic appearance of the cells.

The chronic cases in our series with low blood counts always developed the microcytic type of blood. None showed the macrocytic type such as is occasionally observed in certain of the severe chronic secondary anemias.

Leukemia.—Observations made in eighteen cases of leukemia have led me to the conclusion that in this disease the hematocrit gives only approximate results. Theoretically the leucocytes being lighter should form a column proximal to the column of red cells. This separation of white from red cells does take place to a considerable degree, but the line of demarkation is not sharp. Repeatedly I have found red cells in the inner column and leucocytes deep in the outer column. The higher the white count the more difficult it becomes to take the reading. When the count is below 100,000 the separation is more perfect and the volume index may be roughly estimated.

The great numbers of leucocytes in leukemia also vitiate the color tests for hemoglobin.

Because of these considerable inaccuracies, deductions concerning cell volume must be guarded.

¹ Loc. Citatus.

² Die Anæmie, Wien, 1898.

³ Centralb. f. Stoffw. u. Verdauungskrank, Nr. 11, 1901.

⁴ Loc. Citatus.

The cell measurements and the centrifuge readings in a few instances where the leucocytosis was moderate, indicate that their size is essentially the same as in other secondary anemias.

GENERAL CONCLUSIONS.

1. The centrifuge accurately determines the mass of red corpuscles, but cannot be relied upon to estimate the number of cells, because the volume of the cell undergoes variations in disease.

2. The volume of the individual erythrocyte is best obtained by using the centrifuge in conjunction with the hemacytometer. Volume Index is an expression used to designate the volume of the erythrocyte relative to the normal. Measurement of diameters for the determination of cell size is of limited value and often misleading, especially when poikilocytosis is present.

3. The cell volume is invariably increased in pernicious anemia and usually more so than the Hb content of the cell. This heightened volume index is a more constant and trustworthy sign of pernicious anemia than the increased color index. The polychromemia in pernicious anemia is due to an increase in cell volume and not to an increased affinity of the protoplasm for Hb.

4. The moderate cases of secondary anemia show only a slight loss in cell volume, but if the anemia is of high grade, the cells become small and atrophic.

A small group of secondary anemias exhibits during the early stages the low color and volume indices of a chlor-anemia; later on, as the oligocythemia becomes marked, the blood turns macrocytic with high volume and color values, resembling that of pernicious anemia.

5. In a large proportion of chlorotics the cell volume suffers as well as the Hb, although always to a less extent. The volume index is of great significance in prognosis. The patients who have a normal or nearly normal volume index recover quickly even if the Hb is much deficient. Those who have a low volume index respond slowly to treatment and make a tedious convalescence. The cell volume has

more to do with the rate of improvement than the Hb content, for the cell must grow to normal size before it can take up its full quota of Hb.

6. In all anemias the color loss is greater and takes place more rapidly than the loss in volume. During the process of recovery the volume is restored before the color reaches normal.

7. The Hb content of a normal erythrocyte, as indicated by a color and volume index of 1.00, represents the point of saturation of the protoplasm. When, therefore, the color index rises above 1.00, we may assume that a corresponding increase has taken place in the cell volume (except in jaundice, where the color test is unreliable). Supersaturation of cell protoplasm with Hb probably does not occur. On the other hand, the cell may lose Hb without necessarily losing in volume.

8. Cell volume seems to be chiefly altered by influences affecting cell growth or degeneration. The large erythrocytes of pernicious anemia are probably young cells. Small cells may result from a malnutrition of the bone marrow, as in chlorosis, or from an actual degeneration, as in sepsis.

9. The cell volume suffers remarkably little change from osmotic influences according to my observations. Dropsy, cyanosis, the hydremia following acute hemorrhage, and jaundice (with some exceptions) do not materially alter the volume of the cells.

An explanation of this fact is offered by recent investigations which show that the molecular density of bile and of the lymph fluids present in hydremia is nearly the same as that of blood serum. Osmosis cannot be responsible for the cell alterations in chlorosis, because the shrinkage in cell volume is accompanied by a great loss in Hb. Neither can osmosis be the cause of the increased cell volume in pernicious anemia, for the cell is rich in Hb.

The "acute swellings" described in dropsy, hemorrhage, jaundice, and chlorosis I have never observed.

TABLE I.

Normals.			No. of Red Cells.	Hemat- ocrit.	Color Index.	Vol- ume Index.	Ave. Diam. of Red Cell in μ .
No.	Sex.	Hb					
1	F.	78	4,406,000	45.2	.97	1.02	7.71 μ
2	F.	85	4,676,000	47.0	1.00	1.00	7.70
3	F.	84	4,780,000	47.2	.97	.98	7.64
4	M.	89	5,096,000	49.0	.97	.96	7.62
5	F.	86	4,580,000	48.0	1.03	1.05	7.72
6	M.	90	4,970,000	49.4	1.00	.99	7.60
7	M.	91	5,370,000	52.5	.94	.97	7.55
8	F.	82	4,320,000	44.0	1.04	1.02	7.70
9	F.	84	4,830,000	47.5	.96	.98	7.66
10	M.	92	5,200,000	51.0	.97	.98	7.64
Average	86	4,827,000	48.0	.98	.99	7.65

TABLE II.

Case.	Pernicious Anemia.			Hematocrit.	Color Index.	Volume Index.	Ave. Diameter of Reds in Microns.	Poikilocytosis.	Polychromatism.
	Hb.	Sp. Gr.	Number of Red Cells.						
1	30	1.033	828,000	16.6	1.98	2.00	8.26	Extreme	Extreme
2	20	1.031	900,000	11.5	1.22	1.28	8.08	Extreme	Marked
3	36	1.039	1,436,000	20.0	1.38	1.39	7.54	Marked	Marked
4	37	1.045	1,600,000	25.6	1.27	1.53	8.08	Marked	Marked
5	30	1.037	1,225,000	17.6	1.34	1.43	8.22	Extreme	Extreme
6	14	1.030	659,000	8.0	1.17	1.21	8.32	Extreme	Extreme
7	23	1.036	867,000	13.5	1.44	1.50	8.49	Extreme	Marked
8	30	1.039	1,264,000	17.0	1.30	1.34	7.95	Extreme	Marked
9	39	1.043	1,538,000	24.5	1.39	1.59	8.61	Marked	Marked
10	32	1,250,000	18.0	1.40	1.44	8.35	Extreme	Marked
11	40	1.044	1,648,000	21.7	1.33	1.32	8.02	Marked	Marked
12	28	1.043	1,284,000	16.0	1.20	1.25	8.08	Extreme	Marked
13	39	1.045	2,100,000	22.0	1.02	1.05	7.65	Marked	Marked
14	38	1.044	1,860,000	26.5	1.12	1.42	8.50	Marked	Marked
15	63	1.058	3,600,000	35.9	.96	.99	7.60	Moderate	Slight
16	23	1.040	1,210,000	13.0	1.05	1.07	7.80	Marked	Marked
17	15	1.035	746,000	10.5	1.11	1.40	7.94	Extreme	Extreme
18	46	1.046	3,050,000	33.4	.83	1.09	7.80	Moderate	Slight
19	36	1.040	1,345,000	20.0	1.47	1.49	8.10	Marked	Moderate
20	25	1.033	990,000	13.5	1.39	1.36	7.90	Extreme	Marked
21	17	1.034	948,000	12.5	1.05	1.32	8.00	Extreme	Moderate
22	45	1,886,000	26.0	1.32	1.38	8.20	Marked	Moderate
23	56	2,560,000	34.0	1.18	1.33	7.70	Moderate	Slight
24	28	1,132,000	14.6	1.28	1.30	8.10	Marked	Marked
25	30	1.037	1,220,000	20.0	1.31	1.64	7.80	Marked	Marked
26	31	1,564,000	22.5	1.33	1.44	7.90	Marked	Moderate
27	40	1,220,000	19.0	1.50	1.69	8.20	Extreme	Marked
28	26	1.036	920,000	14.8	1.53	1.60	8.10	Extreme	Marked
29	40	1,240,000	22.5	1.20	1.20	7.9	Marked	Marked
30	23	1.038	1,052,000	13.0	1.20	1.24	8.0	Extreme	Marked
Ave.	32	1,453,000	19.4	1.27	1.37	8.03

TABLE III.

Pernicious Anemia during Improvement.				No. of Red Cells.	Hematocrit.	Color Index.	Volume Index.	Average Diameter of Reds in Microns.	Poikilocytosis.	Polychromatism.
Case.	Date.	Hb.	Sp. Gr.							
2 . .	July 25, '99	28	1.029	900,000	16.0	1.70	1.77	9.01	extreme	marked
	Sept. 6, '99	27	1.031	1,008,000	16.0	1.47	1.59	8.00	extreme	marked
	Nov. 5, '99	38	1.038	1,620,000	22.0	1.29	1.35	7.95	marked	mod.
	Jan. 7, '00	49	1.047	2,312,000	26.7	1.16	1.15	7.8	marked	slight
	Mar. 3, '00	65	1.053	3,200,000	37.0	1.12	1.16	7.7	mod.	slight
	June 2, '00	80	1.060	4,210,000	47.0	1.04	1.12	7.84	slight	slight
	Nov. 7, '00	84	1.061	4,350,000	45.0	1.04	1.04	7.75	slight	none
During Decline.										
7 . .	Dec. 17, '00	57	1.052	3,004,000	38.0	1.04	1.27	8.30	mod.	slight
	Mar. 9, '01	36	1.043	1,650,000	19.5	1.20	1.18	8.01	marked	mod.
	Mar. 24, '01	23	1.036	867,000	13.5	1.44	1.59	8.49	extreme	marked

TABLE IV.

Secondary Anemia, Group I.					Hematocrit.	Color Index.	Volume Index.	Ave. Diameter of Red Cells in Microns.	Poikilocytosis.	Polychromatism.
Case.	Sex.	Disease.	Hb.	Number of Red Cells.						
1	M.	Ulcer of stomach .	80	4,340,000	45.6	1.01	1.05	...	None	None
2	F.	Neurasthenia . . .	70	4,520,000	47.5	.85	1.05	7.60	None	None
3	M.	Diabetes mellitj. . .	80	4,624,000	47.0	.95	1.01	...	None	None
4	M.	Diabetes insip. . .	50	4,090,000	36.0	.68	.90	7.23	Slight	None
5	M.	Dyspepsia	78	4,788,000	45.0	.89	.92	7.33	None	None
6	F.	Dyspepsia	65	3,980,000	40.4	.90	1.01	...	None	None
7	M.	Alc. gastritis . . .	74	4,540,000	46.0	.90	1.01	7.60	None	None
8	M.	Malaria	62	4,366,000	38.5	.73	.88	...	None	None
9	F.	Neurasthenia . . .	72	4,220,000	43.8	.94	1.04	7.56	None	None
10	M.	Intest. indigest. .	75	4,944,000	45.0	.83	.91	...	None	None
11	F.	Dyspepsia	62	3,950,000	41.0	.81	1.03	7.6	None	None
12	M.	Influenza	80	4,880,000	48.0	.90	.98	7.7	None	None
13	M.	Neurasthenia . . .	80	4,370,000	48.1	.90	.98	...	None	None
14	F.	Neuritis	73	4,520,000	42.0	.86	.98	7.56	None	None
15	M.	Colitis	75	4,580,000	45.0	.90	.98	...	None	None
16	M.	Gastritis	75	4,450,000	44.0	.93	.99	...	None	None
17	M.	Cancer of stomach .	30	3,572,000	26.0	.46	.73	7.24	Mod.	None
18	M.	Gastritis	78	4,720,000	46.0	.91	.99	...	None	None
19	F.	Neurasthenia . . .	58	3,700,000	37.0	.86	1.06	7.62	None	None
20	F.	Dyspepsia	74	4,240,000	42.5	.96	1.00	...	None	None
21	F.	Intest. Indig. . . .	72	4,340,000	41.2	.91	.95	7.6	None	None
22	F.	Myelitis	72	4,304,000	39.0	.92	.91	...	None	None
23	M.	Cancer of stomach .	75	4,785,000	47.0	.81	.98	...	None	None
24	F.	Neurasthenia . . .	71	4,560,000	45.0	.87	.98	7.64	None	None
25	F.	Epilepsy	64	3,900,000	38.0	.90	.97	...	None	None
26	F.	Dyspepsia	75	4,716,000	46.2	.87	.98	7.58	None	None
27	M.	Lues	65	4,080,000	40.0	.88	.98	...	None	None
28	F.	Phthisis	60	3,880,000	36.8	.88	.95	7.50	None	None
29	F.	Diarrhœa (chr.) .	70	4,000,000	39.0	.96	.99	...	None	None
30	M.	Tuberc. glands . .	57	4,550,000	32.0	.69	.77	...	None	None
31	M.	Influenza	75	4,400,000	45.0	.94	1.02	...	None	None
32	F.	Salpingitis	45	4,080,000	33.0	.60	.81	7.35	None	None
33	M.	Arthritis deformans	64	3,950,000	37.0	.90	.94	7.52	None	None
34	M.	Rhachitis	40	4,720,000	38.0	.47	.81	7.30	Slight	None
35	F.	Rhachitis	62	4,560,000	37.0	.75	.81	7.40	None	None
		Average	70	4,360,000	41.3	.85	.95			

TABLE V.

Secondary Anemia, Group II.										
Case.	Disease.	Date.	Hb.	No. of Red Cells.	Hematocrit.	Color Index.	Volume Index.	Ave. Diameter of Reds in Microns.	Poikilocytosis.	Polychromatias.
1 . .	Ulcerative endocarditis	Nov. 19	38	3,150,000	26.0	.66	.82	7.3	Slight	None
	Ditto	Dec. 3	30	2,448,000	20.0	.67	.82	7.2	Moderate	None
2 . .	Ulcerative endocarditis	Mar. 3	36	3,112,000	25.5	.63	.82	7.5	Marked	Slight
3 . .	Ulcerative endocarditis	Nov. 19	52	3,900,000	34.0	.73	.87	7.6	None	None
	Ditto	Feb. 6	35	3,372,000	22.4	.57	.66	7.45	Slight	None
4 . .	Acute sepsis		14	1,350,000	8.0	.57	.59	6.4	Marked	Slight
5 . .	Acute sepsis		15	1,500,000	9.0	.55	.60	6.2	Marked	Slight
			28	2,573,000	20.7	.60	.72	7.09		

TABLE VI.

Sec. Anemia, Group III.											
Case.	Sex.	Disease.	Hb.	No. of Red Cells.	Hematocrit.	Color Index.	Volume Index.	Ave. Diameter of Reds in Microns.	Poikilocytosis.	Polychromatolem.	Duration of Disease.
1	M.	Chron. gastritis . .	50	2,350,000	31.0	.93	1.01	7.68	Slight	None	5 yrs.
2	F.	Scurvy	34	2,656,000	24.7	.70	.92	7.44	Slight	None	3 "
3	M.	Lues	25	1,650,000	13.5	.82	.82	7.28	Mod- erate	None	7 "
4	M.	Interstitial nephritis	13	1,840,000	14.0	.39	.76	7.29	Mod- erate	Slight	3 "
5	M.	Addison's disease .	34	2,996,000	24.0	.63	.80	7.30	Mod- erate	None	2 "
6	F.	Nephritis	52	3,000,000	29.0	.92	.94	7.50	Slight	None	4 "
7	M.	Cancer of stomach .	28	2,175,000	18.7	.71	.86	7.35	Mod- erate	Slight	2 "
8	M.	Splenic anemia . .	22	2,170,000	18.5	.56	.85	7.46	Slight	None	2 "
9	M.	Splenic anemia . .	48	2,552,000	27.0	1.03	1.06	7.60	Mod- erate	Slight	8 "
10	M.	Splenic anemia . .	37	1,700,000	21.0	1.20	1.22	7.70	Marked	Marked	5 "
11	M.	Uncinarias	11	748,000	9.1	.38	1.22	7.85	extreme	Marked	2 "
		Averages	33	2,220,000	21.0	.80	.95	7.50			

TABLE VII.

Case.	Chlorosis, Group I.			Hematocrit.	Color Index.	Volume Index.	Ave. Diameter of Reds in Microns.	Poikilocytosis.	Polychromatism.
	Sex.	Hb.	No. of Red Cells.						
1	F.	60	4,480,000	45.0	.73	1.00	7.57	None	None
2	F.	70	4,706,000	42.5	.81	.90	7.45	None	None
3	F.	68	4,600,000	44.0	.81	.95	None	None
4	F.	64	4,390,000	40.0	.80	.91	None	None
5	F.	58	4,582,000	41.0	.69	.90	7.5	None	None
6	F.	74	4,748,000	46.0	.86	.97	None	None
7	F.	70	4,510,000	42.5	.85	.94	7.56	None	None
8	F.	65	4,200,000	41.5	.85	.94	None	None
9	F.	58	4,684,000	37.0	.68	.79	7.38	None	None
10	F.	55	4,140,000	35.0	.72	.88	7.44	None	None
11	F.	51	3,970,000	35.0	.71	.88	None	None
12	F.	68	4,460,000	42.5	.84	.95	7.6	None	None
Ave.	. . .	63	4,450,000	41.0	.78	.92			

TABLE VIII.

Case.	Chlorosis, Group II.			Hematocrit.	Color Index.	Volume Index.	Ave. Diameter of Reds in Microns.	Poikilocytosis.	Polychromatism.
	Sex.	Hb.	Number of Red Cells.						
1	F.	40	4,622,000	36.5	.48	.79	7.09	Moderate	None
2	F.	48	4,070,000	36.0	.65	.88	7.45	Slight	None
3	F.	36	4,160,000	26.0	.47	.62	7.12	Slight	None
4	F.	20	2,200,000	14.0	.50	.64	6.99	Marked	Slight
5	F.	36	3,832,000	33.0	.51	.85	7.23	Moderate	None
6	F.	40	3,874,000	28.0	.56	.72	6.85	Slight	None
7	F.	35	3,100,000	20.0	.64	.65	6.97	Moderate	Slight
8	F.	32	3,120,000	20.5	.57	.66	7.00	Moderate	None
9	F.	35	3,840,000	29.8	.49	.78	7.20	Moderate	Slight
Aver.	. . .	36	3,713,000	28.2	.54	.72	7.10		

TABLE IX.

Chlorosis.				Hematocrit.	Color Index.	Volume Index.	Ave. Diameter of Reds in Microns.	Poikilocytosis.	Polychromatism.	Remarks.
Case.	Date.	Hb.	No. of Red Cells.							
I.	Oct. 17, 1900	60	4,480,000	43.0	.73	.98	7.56	None	None	Ambulatory treatment
	Dec. 13, 1900	68	4,686,000	45.0	.80	1.00	7.57	None	None	Improving
	Jan. 9, 1901	77	4,610,000	47.0	.92	1.02	7.63	None	None	Improving
	Feb. 14, 1901	82	4,700,000	46.8	.96	1.00	7.62	None	None	Seems well
	Apr. 25, 1901	81	4,800,000	48.0	.92	1.00	7.60	None	None	Seems well
II.	Mar. 2, 1901	48	4,070,000	36.0	.65	.88	7.45	Slight	None	Ambulatory treatment
	Mar. 28, 1901	58	4,000,000	39.0	.80	.97	7.5	None	None	Improving
	Apr. 25, 1901	56	4,052,000	37.0	.76	.91	. . .	None	None	Worse. Neglected treatment
	May 16, 1901	64	4,100,000	40.0	.86	.97	. . .	None	None	Better
	June 13, 1901	72	4,340,000	42.0	.91	.97	. . .	None	None	Feels well
III.	Nov. 20, 1900	36	4,160,000	26.0	.47	.62	7.12	Slight	None	Ambulatory treatment
	Dec. 13, 1900	36	4,124,000	29.1	.48	.70	7.38	Slight	None	Improving
	Jan. 10, 1901	42	4,136,000	32.4	.56	.78	7.50	None	None	Improving
	Mar. 21, 1901	56	4,560,000	40.5	.67	.88	7.52	None	None	Improving
	May 3, 1901	60	4,650,000	42.0	.71	.90	. . .	None	None	Improving
	July 7, 1901	64	4,500,000	43.0	.78	.96	. . .	None	None	Improving
	Nov. 27, 1901	84	4,625,000	46.0	1.00	.99	7.60	None	None	Improving
IV.	Oct. 19, 1902	35	3,100,000	20.0	.64	.65	6.97	Moderate	Slight	Bed treatment
	Oct. 28, 1902	40	3,200,000	28.0	.69	.88	7.25	Moderate	None	Improving
	Nov. 8, 1902	46	3,606,000	33.2	.70	.90	7.2	Slight	None	Improving
	Dec. 20, 1902	60	4,200,000	40.2	.78	.96	7.5	None	None	Improving
V.	Nov. 1, 1900	20	2,200,000	14.0	.50	.64	6.99	Marked	Slight	Bed treatment
	Nov. 29, 1900	28	2,932,000	22.0	.50	.76	7.29	Moderate	None	Improving
	Jan. 15, 1901	50	3,800,000	36.0	.72	.92	. . .	Slight	None	Improving
	Mar. 30, 1901	72	4,250,000	41.0	.94	.96	7.5	None	None	Improving

TABLE X.

Nephritis with Dropsy.					Hematocrit.	Color Index.	Volume Index.	Average Diameter of Reds in Microns.	Poikilocytosis.	Polychromatism.
Group I.			Sp. Grav.	No. of Red Cells.						
Cases.	Dropsy.	Hb								
1 . .	Very marked . .	64	1,044	4,080,000	37.0	.86	.91	7.00	None	None
2 . .	Marked	74	1,050	4,062,000	38.0	1.00	.94	. . .	None	None
3 . .	Marked	52	1,040	3,100,000	29.0	.92	.94	7.40	None	None
4 . .	Very marked . .	74	1,059	4,960,000	50.0	.82	1.01	7.7	None	None
5 . .	Marked	85	1,060	5,330,000	52.0	.88	.98	. . .	None	None
6 . .	Very marked . .	70	1,054	4,376,000	41.0	.86	.94	7.5	None	None
7 . .	Marked	80	1,060	4,298,000	42.5	1.02	1.00	. . .	None	None
8 . .	Marked	46	1,050	3,054,000	28.5	.82	.98	7.46	None	None
9 . .	Extreme	60	1,051	4,370,000	42.0	.73	.96	7.23	None	None
Averages		67	1,053	4,160,000	40.0	.88	.96	. . .		

Group II.										
1 . .	None	52	1,052	3,696,000	34.4	.77	.98	7.35	Slight	None
2 . .	None	36	1,044	3,310,000	25.5	.60	.77	7.33	Slight	None
3 . .	None	76	1,059	4,660,000	48.5	.90	1.04	. . .	None	None
4 . .	None	68	1,057	4,380,000	41.5	.85	.96	. . .	None	None
5 . .	None	13	1,035	1,840,000	14.0	.39	.76	7.29	Moderate	Slight
6 . .	None	55	1,056	3,400,000	32.0	.89	.94	. . .	None	None
Averages		50	1,050	3,547,000	32.6	.73	.90	. . .		

TABLE XI.

Jaundice. Group I.				Hb.	Sp. Gr.	No. of Red Cells.	Hematocrit.	Color Index.	Volume Index.	Average Diameter of Reds in μ microns.	Poikilocytosis.	Polychromatium.
Case.	Duration.	Degree of Icterus.	Disease.									
1.	3 wks.	Mod.	Catarrhal Jaundice.	75	1.057	4,920,000	48.0	.84	.98	. . .	None	None
2.	5 "	Mod.	" "	80	. . .	5,320,000	51.0	.83	.96	. . .	None	None
3.	2 "	Mod.	" "	84	. . .	4,630,000	46.0	1.00	.99	. . .	None	None
4.	3 "	Marked	" "	80	1.059	4,220,000	43.0	1.03	1.00	7.70	None	None
5.	4 "	Marked	" "	75	1.057	4,400,000	44.0	.94	1.00	7.65	None	None
6.	3 "	Marked	" "	85	1.060	4,500,000	46.0	1.04	.98	. . .	None	None
Group II.												
7.	5 wks.	Mod.	Gall Stones . . .	60	1.053	3,850,000	35.0	.85	.91	7.00	None	None
8.	4 "	Deep	" " . . .	56	1.048	3,440,000	29.9	.89	.87	. . .	None	None
9.	3 "	Deep	" " . . .	76	1.058	5,160,000	49.2	.81	.95	7.72	None	None
10.	6 "	V. deep	" " . . .	80	. . .	5,000,000	49.5	.88	.99	. . .	None	None
11.	6 "	Deep	" " . . .	85	. . .	5,080,000	51.0	.81	1.01	7.62	None	None
12.	7 "	V. deep	" " . . .	78	. . .	4,530,000	44.0	.94	.97	. . .	None	None
Group III.												
13.	8 mos.	Deep	Cancer of Liver .	60	1.054	3,423,000	33.8	1.02	1.05	7.66	None	None
14.	4 "	Deep	" " " .	72	1.053	3,960,000	38.0	1.00	.96	7.60	None	None
15.	5 "	V. deep	" " " .	72	1.057	3,856,000	36.5	1.05	.95	7.58	None	None
16.	2 "	V. deep	" " " .	68	. . .	4,500,000	42.5	.83	.94	7.45	None	None
17.	6 "	V. deep	" " Pancreas	72	1.054	4,000,000	40.0	1.00	1.00	7.63	None	None
18.	10 "	V. deep	" " Liver .	45	1.048	2,240,000	26.5	1.10	1.18	7.85	None	None
Averages				72	. . .	4,284,000	41.8	.94	.98

TABLE XII.

Case.	Cranosis.		Hb.	Sp. Gr.	No. of Red Cells.	Hematocrit.	Color Index.	Volume Index.	Average Diameter of Reds in Microns.	Poikilocytosis.	Polychromatism.
	Degree.	Disease.									
1.	Mar.	Hepatitis	94	1.067	5,600,000	57.5	1.01	1.03	7.62	None	None
2.	Mar.	Nephritis	80	1.060	4,140,000	42.5	1.03	1.02	7.7	None	None
3.	Mod.	Lues	93	1.063	5,680,000	55.0	.90	.97	7.56	None	None
4.	Mod.	Influenza	84	1.061	5,340,000	52.5	.86	.98	. . .	None	None
5.	Mar.	Chr. Gastritis . . .	57	. . .	3,690,000	38.0	.83	1.03	7.4	None	None
6.	Mar.	Nephritis	85	1.060	5,340,000	52.0	.88	.98	. . .	None	None
7.	Mar.	Mitral Stenosis . .	76	1.060	4,004,000	48.0	.85	.98	. . .	None	None
8.	Very mar.	Asthma	110	1.070	6,200,000	61.0	.18	.98	7.64	None	None
9.	Mod.	Mitral Stenosis . .	88	. . .	5,280,000	51.0	.91	.97	7.6	None	None
10.	Mar.	Myocarditis	90	. . .	5,600,000	56.0	.88	1.00	7.5	None	None
11.	Mar.	Endocarditis . . .	58	1.049	4,500,000	43.5	.70	.96	7.24	None	None
12.	Mod.	Asthma	92	. . .	5,600,000	57.0	.90	1.02	. . .	None	None
13.	Mod.	Phthisis	78	. . .	5,160,000	46.0	.83	.90	. . .	None	None
14.	Mar.	Neurosis of Heart	90	. . .	6,400,000	62.0	.77	.97	. . .	None	None
Average			84	. . .	5,245,000	51.6	.88	.98

TABLE XIII.

Hemorrhage—Acute, Ulcer of Stomach.				Hematocrit.	Color Index.	Volume Index.	Average Diameter of Reds in Microns.	Poikilocytosis.	Polychromatism.	Remarks.
Date.	Hb.	Sp. Gr.	No. of Red Cells.							
Jan. 20, '01	16	1,190,000	11.2	.74	.94	7.68	None	None	1st hemorrhage and hemorrhage, slight
Jan. 23, '01	15	1.033	1,034,000	9.6	.73	.93	7.38	None	None	
Jan. 26, '01	17	1.034	1,364,000	10.5	.69	.77	6.91	Moderate	Slight	
Jan. 30, '01	18	1.036	1,670,000	12.0	.59	.72	7.00	Moderate	Slight	
Feb. 5, '01	20	1.038	1,933,000	15.0	.57	.77	7.00	Moderate	None	Insufficient nourishment
Feb. 14, '01	22	1.038	2,232,000	17.0	.54	.76	7.12	Moderate	None	
Feb. 20, '01	22	1.039	2,274,000	19.0	.53	.83	7.14	Slight	None	
Mar. 2, '01	28	1.038	2,800,000	19.7	.55	.70	6.68	Slight	None	
Mar. 16, '01	31	1.039	2,832,000	21.0	.60	.74	6.93	Slight	None	
Mar. 28, '01	27	1.038	2,763,000	23.0	.54	.83	7.2	Slight	None	
Apr. 24, '01	29	1.043	3,127,000	21.0	.51	.67	7.1	Slight	None	
Sept. 1, '01	46	1.047	4,160,000	30.0	.61	.72	7.3	None	None	
Oct., 1902	66	4,320,000	41.4	.84	.96	7.5	None	None	

TABLE XIV.

Hemorrhage — Chronic, Severe.			Hb.	No. of Red Cells.	Hematocrit.	Color Index.	Volume Index.	Ave. Diameter of Reds in Microns.	Poikilocy- tosis.	Polychrom- atism.
Case.	Duration.	Disease.								
1	2 weeks	Ulcer of stom- ach	35	2,700,000	25.0	.71	.98	7.46	None	None
2	2 mos.	Ulcer of duo- denum . .	52	3,300,000	30.5	.86	.92	7.40	None	None
3	9 weeks	Hemorrhage from uterus.	46	3,600,000	31.0	.68	.84	7.30	None	None
4	1 month	Sarcoma of hip (after operation) .	44	2,608,000	26.0	.92	1.00	7.6	None	None
"	3 "	Ditto.	34	2,200,000	21.2	.89	.96	7.5	None	None
5	4 mos.	Ulcer of stom- ach	50	3,850,000	35.0	.72	.91	7.5	Slight	None
6	9 mos.	Hemorrhoids	20	2,560,000	17.0	.43	.66	7.20	Marked	Slight
7	2 years	Hemorrhoids	29	3,418,000	24.0	.47	.72	7.13	Moderate	Slight
8	12 years	Hemorrhoids	25	3,320,000	21.5	.41	.65	7.20	Slight	Slight
Averages			37	3,072,000	25.7	.68	.84			

Hemorrhage — Chronic,
Mild.

9	2 years	Ulcer of stom- ach	74	4,748,000	46.0	.86	.97	7.6	None	None
10	3 years	Hemoptysis repeated slight . . .	80	4,800,000	47.0	.92	.98	7.64	None	None

BACTERIUM PYOGENES SANGUINARIUM.

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The description of *Bacterium pyogenes sanguinarium* allies it with *bacterium sanguinarium* described by V. A. Moore (U. S. Dept. Ag.: Bureau of Animal Industry, 1895, p. 196. Chester. A manual of determinative bacteriology, 1901, p. 137). The abscess-producing power of the bacterium here described has led to the interpolation of the descriptive word "pyogenes."

During the first part of the month of November, 1902, one of two rabbits confined in a single small cage showed on its hip a wound apparently caused by a bite. This healed quickly, but was followed by the enlargement of a neighboring lymph gland. This gland continued to enlarge, until in about three weeks it was the size of a hen's egg, and soft and fluctuating. At this time several small glands were noticed in various parts of the body. The rabbit was losing weight and strength rapidly. The rabbit was killed December 1, 1902, and autopsy showed each of the glands to be filled with thick, creamy white pus. Cultures showed in every case a pure culture of the bacillus to be described.

Morphology.—The morphology varies greatly with the age of the culture. The measurements below are from cultures on blood serum at 37° C.

In five or six hours many coccus forms appear, varying from 0.6 m. to 1.0 m. in diameter; bacilli varying in length from 1.0 m. to 5.0 m. and in width from 0.5 m. to 1.5 m.; thread forms—very few in number—up to 20 m. in length and from 0.5 m. to 1.5 m. wide.

In eighteen to twenty-four hours the number of thread forms increases greatly, as does the length of the individual threads, while there are relatively fewer bacilli and coccus forms. Some of the thread forms reach a length of 75 m.

¹ Received for publication Aug. 31, 1903.

or even more. At this time chains of short forms are found which look as though a thread had broken up into shorter elements, for in this chain short forms of perhaps 1.5 m. in length will alternate with forms 15 m. to 20 m. long.

In forty-two to forty-eight hours the number of thread forms found is much less, and there is a return to the short bacilli and coccus forms.

In seventy-two hours there is almost total disappearance of thread forms, and we have bacilli and coccus forms remaining. At six and seven days the morphology is practically the same as at seventy-two hours.

In the blood and pleural and peritoneal exudate the forms are practically all short, with very few thread forms.

Stains. — It stains easily with watery and aniline stains, and is decolorized by Gram's method.

Motility. — Non-motile.

Growth on various media. Gelatine plates. — On gelatine plates the surface colonies appear as small, circular, whitish colonies, moist in appearance. There is no liquefaction. Deep colonies appear as small, white spheres.

Gelatine tubes. — Punctures in gelatine tubes show in about three days a uniform and finely beaded growth extending nearly or quite to the bottom of the puncture. There is no liquefaction. In sugar gelatine there is no gas production.

Agar plates. — On agar plates the surface colonies first appear as punctate, hyaline colonies. These gradually increase in size, and in about three days are about one millimeter in diameter, circular, moist, and somewhat whitish colonies seen by transmitted light. The edges of the colonies are distinctly raised above the general surface of the colony.

Agar tubes. — On agar tubes the same appearances are seen as on agar plates. When streaked, the colonies tend to become confluent, but do not spread widely. The edges of the streaks are raised.

Potato. — On potato the growth is practically invisible, and there is no color production.

Milk. — There is no coagulation of milk, and litmus milk shows no change in color.

Bouillon. — At first, bouillon is uniformly clouded. Later a pellicle is formed which sinks to the bottom.

Blood serum. — On blood serum at 37° C., growth is first observed at about five hours, when the surface appears as if very finely sanded. Later the colonies appear punctate and hyaline. In twenty-four hours they appear as circular colonies not exceeding one millimeter in diameter, convex, moist, and almost colorless. In about two days the colonies become somewhat umbonate, dryer, smooth, and somewhat iridescent. Next a slight sinking of that part of the colony around the central projection occurs, which gradually increases until the colony is umbilicated with or without a small central projection in the depression.



An iridescent film is formed on the water of condensation.

Inspissated egg. — Growth on egg is same as on blood serum.

Gas. — No gas production in sugar media.

Indol. — Indol produced. *Pathogenesis.* — At first intraperitoneal inoculation was fatal in twenty-four hours, but by passage through guinea-pigs the virulence was raised so that intraperitoneal inoculation produced death in twelve hours. The germ was isolated from the heart blood, pleural and peritoneal cavities, and all the organs. The pleural and peritoneal cavities contained a sero-fibrinous exudate with enormous numbers of the bacilli. There was some congestion of the abdominal organs, and the intestines were distended with gas. Subcutaneous inoculation causes within forty-eight hours an area of infiltration. This area increases in size, and after about two weeks is fluctuating and contains very thick pus, creamy white or slightly yellowish in color. There is much infiltration of the surrounding tissue. Smears show few bacilli in the pus, but a pure culture is easily obtained.

SUMMARY.

NAME.	BACTERIUM PYOGENES SANGUINARIUM.
Obtained from.....	Suppurating lymph gland in a rabbit.
Shape and arrangement....	Bacilli of varying length; threads; cocci; chains of short forms.
Staining { <i>a.</i> Watery dyes.	Stained easily and quickly by watery and aniline
reaction { <i>b.</i> Gram	dyes. Decolorized by Gram's.
Capsule	None.
Motility	Non-motile. Flagella not yet demonstrated.
Spore production	Apparently none.
Growth on :	Plates : <i>Deep</i> colonies, small, whitish spheres.
	<i>Surface</i> colonies small, discrete, moist, and whitish.
Gelatine	Tubes : In puncture growth extends quite or nearly to bottom.
	Is finely beaded. No liquefaction.
	Plates : Deep colonies, small, whitish spheres.
	<i>Surface</i> , circular, white, discrete, moist colonies with raised edges.
Agar-agar	Tubes : Single colonies as on plates. When streaked, colonies become confluent. Do not tend to spread much. Streaks nearly transparent, whitish with raised edges.
Potato	Invisible growth. No color production.
Blood serum	Circular, moist, colorless colonies. Edges raised above center.
Bouillon.....	At first uniformly clouded. Later pellicle formed which sinks.
Milk	No coagulation. No change in color of litmus milk.
Best temperature	37° C.
Rapidity of growth.....	First appearance in five to six hours.
Need of oxygen	Aerobic. Facultative anaerobic.
Gas production	None.
Indol production.....	Indol produced.
Action on gelatine	No liquefaction.
Color production.....	No color production.
Pathogenesis	At first fatal in 24° on intraperitoneal inoculation.
	Virulence increased by passage through animals so that it was fatal in 12°.
	Subcutaneous inoculation causes abscess formation.
	Much infiltration of tissue with later necrosis of infiltrated area. Abscess reaches height in two to three weeks.

DESCRIPTION OF PLATE XVII.

PHOTOGRAPHS, DR. S. B. WOEBACH.

All magnified 2,000 diameters.

1. Bacilli in pleural exudate.
2. Six hours' growth on blood serum.
3. Eighteen hours' growth on blood serum.
4. Forty-two hours' growth on blood serum.
5. Three days' growth on blood serum.
6. Six days' growth on blood serum.

THE INFLUENCE OF CERTAIN BACTERIA ON THE COAGULATION OF THE BLOOD.¹

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In some former investigations² I undertook an analysis of some of the factors leading to the coagulation of the blood of different species of animals, especially in its relation to thrombosis and the formation of fibrinous exudate. In a preliminary way we can distinguish three kinds of causes leading to coagulation: (1) conditions present in the liquid containing a fibrinogen; (2) conditions present in the surrounding tissues; and (3) changes produced by substances originally foreign to the organism in which they are present, as for example, bacteria and ligatures.

With regard to the second series of conditions, a specificity of the coagulins present in tissues of different species of animals has been found to exist, in so far as the blood of each species of animal used coagulated more rapidly under the influence of the tissues of animals of its own species, or of the tissues of related animals, than under the influence of the tissues of more distant animals. For instance, we would be able to distinguish by this "biological" method the muscle of a guinea-pig from that of a rabbit. This was proven for the blood and tissues of mammals, birds, reptiles, amphibia, and arthropods. The tissues in contact with the fluid containing a fibrinogen may be the seat of an infection with bacteria. Besides the tissues, the bacteria might also exercise some influence on the fluid. Such an influence would, for instance, have to be considered in cases of peritonitis or pleurisy, where coagulation of the fibrinogen might be followed by the formation of adhesions and subsequently

¹ This research was carried out under a grant from the Research Fellowship Fund of McGill University. Received for publication Oct. 19, 1903.

² Über die Bedeutung d. Blutkörperchen, etc. Virchow's Archiv., clxxiii, 1903.

On the Coagulation of the Blood in its Relation to Thrombosis and Fibrinous Exudate. Montreal Medical Journal, No. 7, 1903.

of strictures. The influence of bacteria would have to be considered also in pneumonia and in bacterial inflammations of the walls of the blood vessels. Bacterial infection has possibly a still further significance for inflammatory processes which take place in the interstices of the connective tissue, in so far as the coagulation of a fluid containing a fibrinogen might be an important factor in the new formation of connective tissue, with the resulting formation of a scar and the accompanying retraction of the fibrous tissue formed. This is suggested by the fact that connective tissue, which usually does not grow into liquids, "organizes" solid bodies like coagula. The influence which bacteria might have on the coagulation of a liquid containing a fibrinogen was clearly shown in a number of experiments of transplanted sarcomata in rats. It was found that if the developing tumor was infected with micro-organisms a coagulation of the cystic fluid frequently took place.

If we consider the circumstances under which the coagulating influence of bacteria would be of pathological importance, it would be necessary to exclude the conditions under which a very marked emigration of leucocytes leading to suppuration takes place. The pyogenic bacteria would, therefore, probably be mainly of importance in infections of a chronic character, or otherwise, if they possess a low virulence.

No records could be found of previous experiments on the local influence of bacteria on the coagulation of the blood. Salvioli,¹ however, reported some experiments in which he injected relatively large quantities of the soluble products of certain bacteria into the blood vessels of animals. He found that the injection of a liquid in which the staphylococcus had grown caused a pronounced decrease in the coagulability of the blood. This fact has been confirmed by Delezenne² who also found that the toxins of the diphtheria bacillus were without effect, but that the toxins of the bacillus pyocyaneus

¹ Berliner Klinische Wochenschrift, 1894.

² Delezenne. Action leucolytique de la peptone. Arch. de Physiol. Normale et Pathologique, 5e serie, x, 1898.

were very effective. The methods employed in the experiments of these investigators do not reproduce the conditions which most frequently prevail in the human body in cases in which we find the presence of coagula. Usually we have to deal with a localized action of bacteria, the soluble products of these bacteria mixing with the surrounding liquid.

Most of the experiments to be described here were made *in vitro*, and reproduce approximately the conditions present in the animal organism at places, where a bacterial infection has taken place and where a liquid containing a fibrinogen is present. The blood plasma of geese was obtained according to the method of Delezenne by introducing a canula into the jugular vein of a goose, carefully avoiding any contact of the blood with the tissue, and immediately afterwards centrifugalizing the blood. The blood thus obtained was diluted ten times with a 0.8 per cent sodium chloride solution. Control experiments showed that goose plasma reacts in this dilution approximately in the same way as undiluted plasma.

The best method of determining the influence of bacteria upon the diluted plasma was found to be as follows: In a series of small sterilized porcelain dishes from three to eighteen drops of bouillon culture of the bacteria to be tested were introduced. To each dish three cubic centimeters of diluted goose plasma were added. The dishes were then closed by sterilized covers. The majority of the dishes were kept at room temperature; in each experiment one or two dishes were put into the thermostat. Simultaneously, experiments were usually carried on in test-tubes containing bouillon cultures of the bacteria to be tested. Most of the bouillon was poured off until about one-quarter to one-half cubic centimeter of it remained. To the remaining fluid three cubic centimeters of goose plasma were added, and the test-tubes were then kept like ordinary culture tubes, either at room temperature or in the thermostat at 35°–37° C. Peptone-free culture media were frequently used in these experiments, because in previous experiments it had been observed that ten to fifteen per cent solutions of Witte's peptone often had an accelerating effect upon the coagulation of the plasma. It was

found, however, that the relatively small amount of peptone present in the ordinary culture media has no, or only a very slight, accelerating influence upon the coagulation. It was therefore possible to use also ordinary culture media containing peptone. Usually a number of bacteria were tested simultaneously so that the strength of their coagulating power could be compared.¹

In such experiments it is necessary to avoid the use of methods which introduce factors which have coagulating action. If the blood plasma is poured into ordinary agar tubes used for slant cultures, the coagulation is hastened without the presence of any bacteria. The large solid surface which under these circumstances comes in contact with the blood plasma and possibly also the chemical character of the material contained in the test-tubes are in themselves factors which have a marked accelerating effect upon the coagulation of the plasma. If, on the other hand, we add to blood plasma small particles of an agar slant culture, we usually do not find any marked coagulating influence of the bacteria. All these difficulties can be avoided by employing the method described above.

So far the following bacteria have been tested :

1. *Staphylococcus pyogenes aureus*.
2. *Streptococcus pyogenes*.
3. *Bacillus coli communis*.
4. *Bacillus typhosus*.
5. *Bacillus pyocyaneus*.
6. *Bacillus prodigiosus*.
7. *Bacillus diphtheriæ*.
8. *Bacillus xerosis*.
9. *Bacillus tuberculosis*.

With most of these organisms three, five, or more series of experiments were made, with the exception of the streptococcus and the tubercle bacillus, each of which was tested in only one series. The main result of these experiments can

¹ In the case of each microorganism tested, all cultures were obtained from one original culture. If variations in the coagulative power of cultures obtained from different places exist, this has yet to be determined.

be briefly stated as follows: The *staphylococcus pyogenes aureus* has a specific influence in causing coagulation of the blood. Bouillon cultures of the *staphylococcus* were much more potent than any one of the other organisms. The typhoid bacillus, the diphtheria and xerosis bacilli, as well as the streptococcus and the tubercle bacilli, were without any apparent effect. The *pyocyaneus*, *prodigiosus*, and colon have an intermediate position — the *pyocyaneus* being usually the strongest, the colon the weakest of these three.

It must, however, be taken into consideration that only a glycerine agar, and not a bouillon culture of the tubercle bacillus has so far been employed. The crusts of the bacilli were scraped off and an emulsion was made with a 0.8 per cent sodium chloride solution. Under these circumstances, the soluble substances found in the bouillon culture were missing. With regard to the bacillus tuberculosis, we can so far only state that a suspension of the bacilli alone is without any marked influence upon the coagulation of the plasma. The streptococcus employed in these experiments did not grow well in the bouillon used, and at present its ineffectiveness might be ascribed to the weakness of the culture. With these two organisms further experiments will have to be made.

Cultures of different ages (from one day to several weeks) were used for these experiments. The age of the culture was not of any essential importance. The *staphylococcus pyogenes aureus* caused coagulation almost invariably after from four to eighteen hours. The slight variations usually depended on the proportion in which the bouillon and blood plasma were mixed. First, a coagulum became visible at the bottom of the dish, which soon extended to the surface, until the whole liquid was coagulated and fixed to the surface of the dish. In most experiments one or two dishes containing the mixture of the *staphylococcus* and the goose plasma were coagulated in four to five hours. The plasma mixed with the bouillon culture of the bacteria classed as ineffective was uncoagulated after two or three days in almost

all cases. The pyocyaneus frequently caused coagulation after eighteen to thirty hours. In some dishes, however, the coagulation was delayed for a long period, or did not take place at all. In the case of this organism some difference was noticeable with regard to the effectiveness of different cultures; bacteria grown in peptone-free bouillon behaved frequently somewhat differently in their coagulation from cultures grown in ordinary bouillon. The bacillus prodigiosus showed similar variations. Frequently, after eighteen to forty hours coagulation took place in dishes containing this microorganism. In some dishes, however, no coagulation took place. Cultures of this organism grown in ordinary bouillon and in peptone-free bouillon not rarely differed in regard to their effect upon the coagulation of the plasma, just as they behaved differently in regard to the production of pigment. It is, however, not yet possible to state how the growth in different culture media affects the coagulating power of these two microorganisms. Under the influence of the colon bacillus coagulation of the blood plasma usually took place later than under the influence of the pyocyaneus or of the prodigiosus.

These results were obtained when the experiments were carried on in small flat dishes. If test-tubes were used, the relative coagulation time remained about the same; the influence of the larger surface of contact was, however, noticeable in this case by the acceleration of the coagulation which took place in test-tubes as compared with the coagulation time in small dishes. In both series of experiments the plasma containing the culture of the staphylococcus was almost invariably the first one to coagulate. In most of the experiments control tests were made in which other foreign bodies, like powdered charcoal or small strips of paper, were added to the blood plasma. Charcoal added to the plasma of the goose blood usually had either a weak accelerating effect or none at all. If, however, foreign bodies of a different character — like pieces of filter paper or a piece of dry agar such as is used for the preparation of the culture media — were added, the acceleration of the coagulation was

usually somewhat more pronounced, though the influence of these substances was not comparable to the action of the staphylococcus.

The action of the bacteria on the coagulation of the blood is, therefore, not the action of an otherwise inert foreign body. The constant difference between the effectiveness of the different bacteria tested would alone be sufficient to prove this conclusion. The accelerating influence of bacteria on the coagulation of the blood was not in proportion to the number of microorganisms contained in equal volumes of bouillon. The number of the bacteria in the cultures of the colon bacillus was, for instance, very large, still the colon bacillus had only a weak effect upon the coagulation of the blood. In a similar way could the reaction of the cultures be excluded as the cause of the resulting coagulation. Mixtures of bacteria and blood plasma, which were tested at different periods before and after coagulation, had usually an alkaline reaction. In control experiments the addition of variable amounts of one-tenth normal NaOH to the blood plasma did not have any accelerating influence upon the coagulation of the blood plasma.

If we consider the rapidity with which the coagulation of the blood usually took place after the addition of the staphylococcus culture, it becomes unlikely that chemical substances, produced by the staphylococcus from parts of the plasma, are the direct cause of the shortening of the coagulation period. It is much more likely that substances pre-formed, either in the fluid of the culture medium or in the cell bodies of the bacteria, cause the plasma to coagulate. Which of these two possibilities is the real explanation remains yet to be determined by tests made with the filtered fluid of bacterial cultures. The fact that by employing pieces of agar on which the staphylococcus had grown not even a local coagulation could be induced seems to indicate that substances which are in solution in the liquid culture medium are the cause of the coagulation of the plasma.

In several experiments the contents of a tube containing a staphylococcus bouillon culture were divided into two parts —

one-half being tested in the ordinary way for its coagulating power, the other being sterilized in the autoclave before being mixed with the blood plasma. The first half induced the coagulation of the plasma in the usual time; the second half had either lost its coagulating power entirely, as was the case in the majority of the tests, or its coagulating power was markedly weakened. On the other hand, such substances as Witte's peptone do not lose their coagulating influence upon blood plasma by being sterilized. It is therefore not unlikely that the bacterial substances causing coagulation have the character of ferments.

The experiments recorded here were all made with goose plasma, and the question now arises whether the results obtained can be applied to mammalian blood. This seems highly probable, for the following reasons: We can distinguish between specific and non-specific coagulins or coagulative substances. To the former belong the normal tissue coagulins; to the latter belong such substances as Witte's peptone (as used *in vitro*), and also certain tissue coagulins, and, to a certain extent, probably ferments present in the blood clots of different species of animals. The non-specific substances causing coagulation act on the blood of both birds and mammals. It is very likely that the bacterial substances causing coagulation belong to the class of non (or less) specific substances, if we consider that specific substances have only been found in animal tissues, and that even here also non-specific substances may exist.

It was found that the blood plasma which had coagulated under the influence of the staphylococcus assumed a whitish, opaque appearance after one or two days. In this way it was easy to distinguish blood plasma which had coagulated under the influence of the staphylococcus from blood plasma which had coagulated under the influence of any of the other organisms tested.

A few experiments were made on the influence of bacteria on goose plasma when injected into the peritoneal cavity of a rabbit or a pigeon. In previous experiments it was found that goose plasma diluted ten times with a 0.8 per cent NaCl

solution does not, after injection into the peritoneal cavity of a rabbit or a pigeon, coagulate inside of the next two hours. Eight cubic centimeters of diluted goose plasma were injected into the peritoneal cavity of a small rabbit, and the same amount into the peritoneal cavity of a pigeon, at three o'clock P.M.; at 3.45 about one cubic centimeter of a several weeks' old bouillon culture of the staphylococcus was injected into the peritoneal cavity of the same animals. Two hours later the blood plasma injected into the rabbit had formed a soft coagulum; it was still uncoagulated in the pigeon. In this case the staphylococcus seems to have caused the coagulation of the blood plasma inside of the animal body.

Salvioli and Delezenne found that injection of a large quantity of the soluble products of the staphylococcus into the veins of an animal caused a decrease in the coagulability of the blood. The soluble products of the bacillus pyocyaneus had a similar effect. These facts seem to be opposed to our results obtained in vitro. The difference, however, is probably only an apparent one. We have here, in all probability, to deal with a phenomenon similar to the one observed in the case of peptone, which has only an inhibitory effect on the coagulation when injected into the veins of certain animals, but which has in vitro an accelerating effect. In the case of peptone the liver is the organ which reverses the effect of peptone upon the coagulation of the blood.¹ The liver plays, perhaps, a similar part in the case of the soluble products of bacteria.

If we consider the large number of fermentative actions exerted by microorganisms, it becomes likely that, at least in the case of some bacteria, their influence on the coagulation of blood is the result of several counteracting processes. We know that proteolytic ferments injected into the circulation of certain mammals have an inhibiting effect upon the coagulation of the blood (Albertoni). In a similar way certain ferments produced by bacteria might inhibit the coagulating influence of other substances produced by the same bacteria. This would, for instance, explain certain variations found in

Delezenne. Archives de Physiologie Normale et Pathologique, 1896.

the effectiveness of the cultures of the bacillus pyocyaneus and prodigiosus.

We may also conclude from our experiments that the fibrin present in membranes in cases of diphtheria is in all probability not formed under the influence of the bacillus diphtheriæ. We may further conclude that under various pathological conditions the staphylococcus pyogenes aureus plays an important part in causing thrombosis and the formation of a fibrinous exudate. Further investigations will have to be undertaken to determine the influence of the pneumococcus upon the formation of fibrin in cases of pneumonia. Under conditions in which thick fibrinous capsules around certain organs are formed (for instance the "Zuckergussleber" of Curschmann and the "Hyaloserositis" of Nicholls), the coagulating influence of certain bacteria may be a causative factor. In this connection also the pronounced action of the staphylococcus pyogenes aureus will probably have to be taken into account.

In conclusion, the records of one of the experiments are here given :

EXPERIMENT XVII.

I. In small dishes (covered) at room temperature, bacteria added to plasma at 4 10 P.M.

(1.) Ten drops of an ordinary twelve days' old bouillon culture of the staphylococcus pyogenes aureus, plus 3 cc. goose plasma diluted 1:10. At 6.30 P.M. all coagulated.

(2.) Six drops of the same culture, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. almost all coagulated.

(3.) Six drops of a fifteen days' old peptone-free bouillon culture of the staphylococcus pyogenes aureus, plus 3 cc. goose plasma. At 6.30 P.M. coagulation begins at bottom of dish; at 8.10 P.M. almost all coagulated; next morning all coagulated.

(4.) Ten drops of the same culture, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; at 8.10 P.M. all coagulated.

(5.) Ten drops of a twelve days' old peptone-free bouillon culture of bacillus pyocyaneus (no pigment developed), plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; at 8 P.M. no coagulation; next morning some coagulation at bottom of dish; second morning one-quarter coagulated.

(6.) Six drops of the same culture, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning almost all coagulated; second morning all coagulated.

(7.) Five drops of a twelve days' old ordinary bouillon culture of *bacillus pyocyaneus* (pigmented), plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning no coagulation; second morning some threads formed.

(8.) Eight drops of the same culture, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning almost all coagulated; second morning all coagulated.

(9.) Ten drops of peptone-free (unpigmented) twenty-two days' old bouillon culture of *prodigiosus*, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning half coagulated, second morning all coagulated,

(10.) Four drops of the same culture, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning no coagulation; second morning half coagulated.

(11.) Ten drops of an ordinary twenty-two days' old bouillon culture of *bacillus prodigiosus* (pigmented), plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning no coagulation; second morning no coagulation. (The *prodigiosus* culture was itself gelatinous. No solid coagulum was formed as in the case of the *staphylococcus*, or in Nos. 9 and 10, but the formation of a very soft coagulum cannot be excluded.)

(12.) Three drops of the same culture, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. some coagulation; 8 P.M. all coagulated.

(13.) Six drops of the same culture; the same result as in No. 11.

(14.) Three drops of a twelve days' old peptone-free bouillon culture of the *bacillus coli*, plus 3 cc. 1:10 goose plasma. At 6.20 P.M. no coagulation; at 8 P.M. no coagulation; next morning no coagulation; second morning coagulation at bottom of dish.

(15.) Ten drops of the same culture, plus 3 cc. 1:10 goose plasma. At 6.30 no coagulation; 8 P.M. no coagulation; next morning no coagulation; second morning no coagulation.

(16.) Six drops of the same culture, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning no coagulation; second morning no coagulation.

(17.) Ten drops of a twenty-two days' old peptone-free bouillon culture of the *bacillus coli*, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning trace of coagulation at bottom; second morning somewhat more coagulation at bottom of dish.

(18.) Six drops of a twelve days' old peptone-free bouillon culture of the *diphtheria bacillus*, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning no coagulation; second morning no coagulation.

(19.) Ten drops of the same culture, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning no coagulation; second morning no coagulation.

(20.) Ten drops of a twelve days' old ordinary bouillon culture of the

bacillus diphtheria, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning no coagulation; second morning no coagulation.

(21.) Six drops of the same culture, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning no coagulation; second morning no coagulation.

II. Small covered dishes containing the mixture of bacteria and plasma put into thermostat (Temp. 35°-37° C.) at 4:10 P.M.

(1.) Six drops of a twenty-two days' old peptone-free bouillon culture of bacillus prodigiosus, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; at 8 P.M. some threads and a little coagulum formed; next morning all coagulated.

(2.) Six drops of a twelve days' old diphtheria bouillon culture, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; at 8 P.M. no coagulation; next morning partially coagulated at bottom. (This was the only case in which a slight coagulation took place in cultures of *Bacillus diphtheriae* in dishes. The higher temperature frequently shortened the coagulation time of the blood plasma.)

(3.) Six drops of a twelve days' old peptone-free pyocyanus bouillon culture, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning almost all coagulated; second morning all coagulated.

(4.) Six drops of a twelve days' old peptone-free bouillon culture of bacillus coli, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning no coagulation; second morning no coagulation.

(5.) Six drops of a twenty-two days' old culture of bacillus prodigiosus, plus 3 cc. 1:10 goose plasma. At 6.30 P.M. no coagulation; 8 P.M. no coagulation; next morning no coagulation; second morning no coagulation.

III. Experiments in test-tube (proportions of cultures: $\frac{1}{4}$ - $\frac{1}{2}$ plus 3 cc. 1:10 goose plasma) mixed at 4.15 P.M.

(1.) Fifteen days' old peptone-free culture of staphylococcus pyogenes aureus. At 8 P.M. almost entirely coagulated; next morning exhibited opaque color of coagulum.

(2.) Twenty-two days' old ordinary bouillon culture of prodigiosus. At 8 P.M. some coagulum; next morning all coagulated.

(3.) Twelve days' old peptone-free culture of pyocyanus. At 8 P.M. no coagulation; next morning some coagulation; second morning coagulation further progressed, not complete.

(4.) Twenty-two days' old peptone-free culture of colon. At 8 P.M. no coagulation; next morning coagulated.

(5.) Twelve days' old ordinary bouillon culture of *Bacillus Diphtheriae*. 8 P.M. no coagulation; next morning no coagulation; second morning two-thirds coagulated.

SUMMARY.

1. In mixing bouillon cultures of bacteria in certain proportions with the diluted blood plasma of a goose, the power bacteria have of coagulating fluids containing a fibrinogen can be tested in vitro and approximately constant results obtained.

2. Among the organisms tested, the staphylococcus pyogenes aureus was found to possess a strong coagulating power, causing frequently coagulation of the plasma in four to six hours. Bacillus Diphtheriae, Bacillus Xerosis, Bacillus Typhosus, Bacillus Tuberculosis, and Streptococcus Pyogenes were without any marked coagulating power. For the last two named organisms, however, this result can at present be accepted only with certain restrictions. Bacillus pyocyaneus, Bacillus prodigiosis, and Bacillus coli have not as strong a coagulating power as Staphylococcus pyogenes aureus; their coagulating power is, however, stronger than that of the second group.

3. The reaction of the cultures is not the cause of their coagulating power. A sterilized culture of the staphylococcus pyogenes aureus has lost all, or a great part, of its coagulating power. The coagulating action of bacteria is not identical with the contact action of an otherwise inert foreign body; it is probable that bacterial products contained in the liquid culture media are the direct cause of the coagulating activity of certain bacteria.

(I wish to express my sincere thanks to Dr. Adams, to whose kindness and interest in these researches I am much indebted for the opportunity of carrying out these and other investigations in the pathological laboratory of McGill University.)

OBSERVATIONS, ESPECIALLY WITH THE ROENTGEN RAYS, ON
THE ARTIFICIALLY DEFORMED FOOT OF THE CHINESE
LADY OF RANK, IN RELATION TO THE FUNCTIONAL
PATHOGENESIS OF DEFORMITY.¹

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A minute examination of the bones of the foot-skeleton of a Chinese lady of rank, collectively and separately, offers an opportunity for interesting study: first, because the forces necessitating change of function are exerted upon a previously healthy organic structure in its normal course of development, and second, because these forces are so applied as to produce results observed similarly in, and in every respect comparable to, no other condition.

Among those who have availed themselves of such opportunity to study the Chinese foot is Hans Virchow,² of Berlin, who has recently made the most careful and exhaustive examination of the abnormalities observed in each bone making up the Chinese foot-skeleton. His monograph is supplemented by accurately made drawings and tracings, and in his description he lays especial stress upon the various facet-deviations. It would be of little value, if not impossible, by a presentation of the subject in a paper of this length, to attempt to make more detailed account than has Virchow in his work. We have found, however, that examination by X-ray of the internal structure of the disarticulated bones, as well as the foot-skeleton as a whole, reveals changes of structural conditions upon which other writers have not laid stress. To present the results of these examinations by X-ray is, therefore, the primary purpose of this paper. A description of the comparative gross appearances is made for purposes of clearness and broadly to explain general topography,

¹ Received for publication Sept. 17, 1903.

² Virchow, H. Das Skelett eines verkrüppelten Chinesinnen Fusses. Zeitschr. f. Ethnologie, Heft 2, 1903.

although we fully recognize the great thoroughness and attention to detail of other writers.

It has been asserted that the organic structure acted upon, which has been bone in most observed cases, plays two parts: on the one hand, a purely passive one, in which it allows itself to become defective or altered in development when opposed to the force-producing source, and develops abnormally in the direction not resisted by, but rather favored by, this applied force. The other part, which has been regarded as a secondary process, is one characterized by a reaction of the organism, shown by various phenomena of functional change. In considering the conditions observed when the organism is playing its passive part, it is very natural to assume that there is some abnormal state of the organism at the beginning, as, for instance, the property of plasticity due to abnormal softness of bones,¹ or that, as stated above, healthy bone may be directed by pressure and may grow in the line or direction of least resistance. The law of functional adaptation as laid down by Wolff² is, of course, not in agreement with the theory of the purely passive attitude assumed by the organism, although he recognizes this as a partial phenomenon of certain pathological conditions.

Perthes,³ in an admirable paper describing his observations on the changes in the Chinese foot, carefully considers each theory that has been advanced with regard to the genesis of the weight-deformities of bone structure. He comes to the conclusion that these changes result from two distinct processes (above mentioned); to wit, a process of morphological adaptation, and a reactive process of functional adaptation.

The clinical observations of Perthes and of Vollbrecht,⁴ especially those made in China, have, probably, not been equalled by any other writer, embracing, as they do, in the experience of Perthes, the examinations of no less than eleven

¹ Mikulicz. Die seitlichen Verkrümmungen am Knie and deren Heilungsmethoden. Arch. f. klin. Chir., Bd. xxiii.

² Wolff. Die Lehre von der functionellen Pathogenese der Deformitäten. Arch. f. klin. Chir., Bd. liii, H. 4.

³ Perthes, G. Über den künstlich missgestalteten Fuss der Chinesin im Hinblick auf die Entstehung der Belastungsdeformitäten. Arch. f. klin. Chir., Bd. lxvii, H. 3.

⁴ Vollbrecht. Der künstlich verstümmelte Chinesenfuss. 1900.

persons from fourteen to twenty years of age, and a twelfth case at the age of forty. From these cases casts, photographs, sole impressions, and X-ray photographs were made, and a series of valuable statistics drawn up. Perthes, however, deplotes his inability to obtain a foot from a Chinese female cadaver, owing to the peculiar superstitious views of the Chinese, and this insurmountable difficulty was likewise met with by Vollbrecht in the pursuance of his investigations in China. It has been our extreme good fortune to obtain two well-preserved specimens of the bony structure of the Chinese foot, one disarticulated and another in which the bones of the tarsus are bound together by their ligamentous attachments in the original position of the deformity. These specimens, originally the property of Dr. Buckminster Brown, as a part of his monumental orthopedic collection, are now in the possession of Harvard University, in the Warren Museum. The observations of Virchow and Heberer¹ were likewise made from examinations of the actual disarticulated bones, the possession of which alone made their careful work possible. The observations of Perthes, by means of the material and methods which he had at hand, were most skilfully and thoroughly pursued. The most accurate of these methods, for purposes of minute examination of changes in bone structure, were the photographs of the living foot-skeleton made by the X-ray. Even this method, however, dealing as it did with shadows thrown upon a plane, was obviously attended by many inaccuracies in the comparative measurements of the bones, which was mentioned as a fact by Perthes.

By means of the exceptional material at our command, we have been able to make both solar and X-ray photographs of the several disarticulated bones of the Chinese tarsus for comparison with photographs, similarly made, of the bones of the normal female foot. Virchow, in employing a similar method of comparison, uses a full-grown male foot skeleton as the subject for his reproductions of the normal, in the belief that, in so doing, the contrast would be made more

¹ Heberer. Schädel und Skeletteile aus Peking. Jena, 1902.

striking. It is our impression, however, that a more conservative comparison can be made by using bones of the same sex. Being thus unimpeded by the presence of the soft parts of a living subject, or by difficulties in X-ray technic from imperfect photographic impressions due to the elevation of the skeleton from the plate, or movement on the part of the subject, it has been possible to examine clearly and minutely the changes which have taken place in both the outer and inner structure of the bones, which are later to be described in full.

It is necessary merely to mention the method employed by the Chinese in the production of this deformity in the feet of their young girls who are destined, as women, to become members of the highest social rank. The custom consists in the application of cotton bandages to the feet of young girls from five to eight years of age, so that pressure will be exerted in two directions. In the first direction, the posterior part, embracing the heel, and the anterior portion of the foot are gradually drawn together, so that they eventually meet and coincide with each other at the mid-tarsus (*vide* Plate XVIII., showing a plaster cast of the bandaged foot, as above described). The other part of the bandaging process is rotatory in direction, and by pressure thus exerted the metatarsals, from the second to the fifth, are drawn inward and underneath toward the first metatarsal, which remains in its normal position. This process of bandaging and its clinical effects are exhaustively described by Perthes, and further account of it is unnecessary for the purposes of this paper. It is desirable to emphasize the fact, however, that the Chinese binding process is by no means an acute, painful procedure, but rather a slow and gradual, but never yielding, moulding of the foot-skeleton to the last extremity of deformity. This fact is likewise enforced by both Virchow and Perthes.

A photograph (Plate XIX.) of the bones and ligaments of the Chinese female foot, when the process of bandaging is completed, shows the nature of the deformity. The usual mild declivities of the arch-slopes of the normal foot are here

exaggerated into a condition of intense *pes cavus*. The abnormal position may best be seen grossly in the almost vertical position of the calcaneum, as well as in the steep slope of the dorsum of the foot. Thus it may easily be noticed from the photograph that the bulk of the body weight-bearing, as transmitted by the leg, is assumed by the calcaneum in the direction of its long axis. The weight-bearing function of the heads of the first and fifth metatarsals is practically obliterated.

In order properly to discuss the various theories advanced by certain writers as to the laws governing the production of static weight deformity, it is necessary to examine accurately not only the gross appearances of the Chinese tarsal bones, but their inner structure as compared with those of the normal foot. In work of this character, where an observer is seldom able to obtain more than one or two specimens upon which to base his opinions, and in which are seen the results produced by the variable procedures of man in opposition to the stable laws of nature, it is possible only to describe that which one himself sees, and it is both unwise and unreasonable to attempt to refute the observations of others. It is possible that one observer may make out, from one or two examples, abnormalities peculiar to the individual from which the specimens were taken. Such abnormalities may not have been recognized by another observer, and conditions which another describes may not exist in the specimens which one has before him. However, as has been stated above, the general picture of deformity is so nearly the same in all cases as to warrant the condition to be an independent one irrespective of the details of individual observations.

While agreeing with Perthes that processes of two natures exist in the development of the foot of the Chinese woman, namely, passive changes of form under the influence of abnormal weight-transmission, and reactive processes of functional adaptation, and also, while recognizing the great ingenuity of the classification of Virchow of the changes observed by him into six great divisions, it seems to us necessary to add another change or process. This additional

change to be found in the Chinese foot-skeleton may be likened to the phenomena seen in bones which are subjected to any irritative influence from outside. It is certainly easy of belief that such irritative influences exist in a condition where inter-relational changes are produced between bones by such powerful and overwhelming outside influences, when we are certain of their presence in such modified conditions as, for example, the various shoe-deformities seen clinically.

As a factor in causation, we cannot here apply the theory of Mikulicz of purely passive bone-conformation under weight, which he likens to the changes seen in certain rachitic deformities, because in the Chinese no previous pathological condition necessarily obtains. We agree with Perthes, nevertheless, that the bones of the Chinese foot play partly a passive role, together with functional adaptation in structure. Our observations on the disarticulated bones have led us to believe that, besides this reactive part of functional adaptation, there exists also a condition, equally reactive, due to the effect of general or localized irritation. The effect of this reaction to irritation may be observed on the external surfaces of certain bones by the presence of various "spurs" and exostoses. Nothing of osteophytic formation Virchow declares to have been seen in his specimens.

Since far more is to be seen in the separated tarsal bones than in the foot-skeleton as a unit, it may be well briefly to describe those separated bones most concerned in the deformity and those most involved in the production of morphological change.

The Calcaneum. — This bone (Plates XX. and XXI.) presents, perhaps, more noticeable change in conformation and structure than any other bone or group of bones in the foot-skeleton. This may be due to the fact that it supports all, or nearly all, of the body-weight. As it is approximated toward the medio-tarsus by the anteroposterior compression bandages, its long axis, instead of being directed forward and outward, is placed vertically, or nearly so, according to the thoroughness

of the bandaging process. The rotatory wrapping shows its effects when we look at the bone from behind (Plate XXI., Fig. 2). Here can be seen an inward bowing of the vertical bone-shaft, which in our specimen was more marked than the usual appearance of the normal calcaneum. Considering, however, what a large proportion of the deformity-producing force is directed against this bone, it is surprising, as Virchow says, to observe to what a degree it retains its normal form. The plane of the long axis is, accordingly, at a different angle with the plane of the superior articulating surface (Plate XX., Figs. 3 and 4. Compare length of line CD with line C'D' in relation with length of lines AB and A'B'. Compare also angle AED with angle A'E'D'). This articulating surface possesses a lesser area as a whole, as compared with the length of the bone, than is found in the normal (Plate XXI., Figs. 3 and 4. Compare lines VX and YZ with lines V'X' and Y'Z'). The posterior portion of the superior surface found in the normal bone is lost in the calcaneum of the Chinese foot and is to be recognized here, forming a part of the posterior surface. The two or three facets usually made out on the superior articulating surface of the normal bone are recognized only with difficulty in the Chinese calcaneum (Plate XXI., Figs. 1 and 2). Concerning the condition of the upper joint facets of the calcaneum, therefore, the observations on our specimen do not agree with those of Virchow, who found them to be very much less modified.

The ordinarily well-marked facet for articulation with the cuboid is also much modified and somewhat obliterated (Plate XX., Figs. 5 and 6). Our observations here coincide in every respect with those of Virchow, who describes an oblique position of the facet in question, *i.e.*, inclined plantarwards instead of maintaining its usual position. In addition, we have found this facet to be modified in structure, rough and unusually irregular of surface. The proportions of the various diameters are changed, as Virchow has pointed out.

On both specimens of the Chinese calcaneum under our observation were found well-marked exostotic "spurs" on the posterior surfaces, as well as overgrowths of the same

nature about the facets on the superior surface of one of them (Plate XX., Fig. 2. Plate XXI., Fig. 2). The external facet on the superior articulating surface of the Chinese calcaneum is the only one that is not markedly modified.

The Astragalus. — The astragalus (Plate XXII.) of the Chinese foot presents moderate changes only, except, in our specimen, the facet for articulation with the *sustentaculum tali* on the os calcis. This facet presents such sharpness of outline in the normal bone that any modification of its appearance, as is seen in the Chinese bone, is very noticeable. The head and neck of the astragalus, as was observed by Perthes, are bent downward in relation to the body, that is to say, pointing downward and forward to conform with the steep inclination of the dorsum of the foot (Plate XXII., Figs. 3 and 4). Between the head and neck superiorly there is a well-defined bony ridge, irregular and roughened, which may be considered analogous to exostoses seen elsewhere (Plate XXII., Fig. 4).

The Scaphoid. — On the navicular bone the most marked peculiarity to be seen is an elongation, in the plantar direction, of the facet for articulation with the head of the astragalus (Plate XXIII., Fig. 2, Point A). This is, without doubt, due to the modified inter-relation of these bones consequent upon the high arching of the mediotarsus. This elongation of the facet changes the disposition of its area in relation to the proximal surface of the bone, especially at the inner side of the foot. The inferior surface of the bone, also at the inner side, presents three or four spurs, seen plainly in the photograph.

The Cuneiforms. — The proximal facets on each of these bones, for articulation with the scaphoid, present a similar elongation plantarwards. The dorsal portion of these facets, which would be seen clearly enough normally, have disappeared, and are supplanted by roughened, attenuated surfaces. In this we find a good example of the phenomenon aptly described by Virchow as "joint-wasting"¹ which he explains as being an actual obliterating bony change which

¹ Verödung von Gelenkabschnitten.

takes place at those parts of a joint rendered functionless by the deformity-producing source. The relation between the internal and middle cuneiform bones seen in one of our specimens was more striking than any other phenomenon of bony change. These bones were found to be firmly fused together by a bony band at their facet for articulation. The non-articulating surfaces, as well as the edges around the facets, were found to be roughened and irregular on both bones (Plate XXIII., Fig. 5).

The Cuboid. — On the cuboid the facets for articulation with the fourth and fifth metatarsals are changed in that their planes are directed downward instead of directly forward. The articulating surfaces occupy only about half of the area in relation to the bone as do those that are seen normally (Plate XXIV., Figs. 1 and 2). The normally smooth oval facet for articulation with the external cuneiform is obliterated and its place taken by rough, irregular, bony structure (Plate XXIV., Figs. 3 and 4).

The Metatarsals. — In the Chinese foot these bones present peculiarities most marked in the relation of shaft and extremities. It may be easily seen from the illustration that the size of the extremities of these bones is, to a great degree, out of proportion to the diameter of the shaft at any given point. By comparing the photograph of Metatarsal V. from the Chinese foot with the corresponding bone of the normal, morphological change is made clearly evident (Plate XXIV., Figs. 5 and 6). Virchow has exhaustively explained these appearances on the ground of compression-atrophy and atrophy from non-use. The soundness of this theory may be easily recognized by a comparative examination of the bones in the accompanying illustration.

The results of our examinations of the separated bones of the Chinese foot-skeleton have been to bring to the attention the presence, as grossly described above, of several types of local hypertrophies; to wit, spurs, general roughness of surfaces, modified new facets for false articulations, and enlargements at and about original articular surfaces. To what

are these changes due, and what relation do they bear to the genesis of weight deformity of bone structure?

The theory of "joint-wasting" of Virchow to our mind accounts for many of the alterations at joint-faces which, through the deformity, are no longer functionally useful, but the appearances which he explains as the result of this process, or of the other processes of his nomenclature, are not the only ones to be observed on our specimens, as has been stated above.

From what has been described with regard to the altered morphology of the Chinese foot as the result of tight bandaging, it cannot but be admitted that the effect of this procedure must be regarded as highly irritative, especially as the process is long-continued and gradual. By virtue of its histological situation, no structure in the Chinese foot can more readily show the effects of long-continued irritation than the periosteum. This irritation gives rise to the proliferation of periosteal osteophytes which are deposited on the bone with resulting exostosis and apposition; with reference to these processes, the attention is again called to the relation between the external and middle sphenoid bones of the Chinese foot. Between these bones fusion has taken place by the bridging over of their articulations, rather than by any ankylotic apposition of the articular surfaces. The results of these investigations, therefore, are not to deny the theories already brought forward as to the presence of a passive process of morphological adaptation, or a reactive process of functional adaptation, but rather, in addition, to recognize the existence of a pathologically reactive process to external irritation which must of necessity take by no means a minor part in the pathogenesis of deformity.

Concerning the inner structure of the Chinese foot-bones (Plate XXV.), if comparative X-ray photographs are made of the separated structures (Plate XXVI.) much is to be observed. Perthes was prompt to take advantage of Roentgen-ray examinations of the exceptional material under his control. He was, however, confronted by the many difficulties of examining minutely bone structure in the living

subject, as has been stated above. Our examinations of the separated bones by this method, together with those of the corresponding bones of the normal foot, we have endeavored to conduct with the utmost care as to the placing of the specimens in relation to our source of Roentgen illumination.

Perthes describes three groups of structural "beams" or "rafters" in the human foot-skeleton. The first group of beams may be traced by the placement of the bone-trabeculæ from the astragalo-tibial articulation in a series of curved lines downward and backward to the tuberosity of the calcaneum. Group II. runs from near the origin of Group I. downward and forward through the mediotarsus to the foretarsus. Group III. sweeps forward from the posterior surface of the os calcis to the inferior surface of the mediotarsus. Our X-ray examination of the normal foot-skeleton verified the general directions of these groups of beams as indicated by the disposition of the trabeculæ. They do not bear out the statement of Perthes, however, that the structural changes and consequent abnormal weight-bearing function of the Chinese foot-skeleton cause a more or less regular re-disposition of the weight beams. If examination be made of the comparative X-ray photographs (Plate XXVI.) of the various bones of the tarsus, it will be seen that the trabeculæ in the Chinese bones are irregularly distributed, without regard to new weight-bearing beams, definitely re-disposed. The instance where definite redistribution seems to have taken place is in the long weight-bearing axis of the calcaneum, shown by a gathering together of weigh-beams at a point where especial resistance may have been most needed. This disturbance of trabecular distribution can be observed in all the photographs made by X-ray, an examination of which will better explain the appearances than printed words in the text. In figures one and two (respectively, the calcaneum and the astragalus) the above-described phenomena are clearly evident. The astragalus presents especially well the picture of trabecular disorder. A metatarsal and the scaphoid are both included in figure three. There is here to be noted the great relative thickness of the cortex of the

shaft of the metatarsal. The spurs on the navicular bone above described are here seen, and present the same degree of radiability as do like conditions seen clinically. Figure four presents the fused cuboid and external sphenoid which we mentioned to have existed in one of our specimens.

From what has just been observed, it is easy to realize what minute knowledge might be gained by making and examining thin sections of these bones, if it were but possible to sacrifice so valuable a specimen for the purpose. Equally valuable work would be accomplished, as Virchow has pointed out, in making radiographic records of the Chinese foot seen clinically in the shoe and out of it, and of the behavior, in its various modified functional processes, of this highly deformed structure. Here the radiologist has a future field in which to obtain exact and satisfying results.

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DESCRIPTION OF PLATES.

PLATE XVIII. Plaster cast of the Chinese foot as seen clinically.

PLATE XIX. Chinese foot-skeleton, showing the bones in situ, with their ligamentous attachments.

PLATE XX. The Chinese calcaneum seen laterally and from in front, in comparison with the norm.

PLATE XXI. The Chinese calcaneum seen from behind, in comparison with the norm.

PLATE XXII. The Chinese astragalus, seen in two aspects, in comparison with that of the normal tarsus.

PLATE XXIII. Comparative photographs of the normal and Chinese cuboid (seen from in front and laterally) and metatarsal.

PLATE XXIV. The scaphoid of the normal and the Chinese foot, showing its facet for articulation with the astragalus. The Chinese internal and middle cuneiforms seen fused together.

PLATE XXV. Radiograph of the Chinese tarsus in the lateral position.

PLATE XXVI. Comparative radiographs of the normal and Chinese calcaneum, astragalus, navicular, and metatarsal, as well as the internal and middle cuneiforms (separated in the norm, fused in the Chinese).

METHODS OF TREATMENT OF CONGENITAL DISLOCATION OF THE HIP.¹

E. H. BRADFORD, M.D.

Success of a surgical procedure must be based on sound pathology. The early attempts in the treatment of congenital dislocation of the hip failed, as the pathology of the affection was not understood. As the deformity is understood now, it is evident that treatment by bed traction, even if continued for years (the method first recommended in France in the middle of the 19th century and afterward revived in this country), would fall short of permanent success. In the resistant cases no endurable amount of traction would overcome the shortened tissues, and even if the head of the femur were pulled down opposite its normal position, massage or passive motion would be insufficient to bore out an acetabulum through the thickened layers of capsule interposed between the head of the femur and the socket. The strength and nature of the contraction were not known and the condition of the capsule covering the acetabulum and stretched around the head was not known. The method failed absolutely.

As antiseptic surgery made operative interference relatively safe, attempts were made to cure the deformity by operation. Excision of the head of the femur was unfortunately performed by a few surgeons, impelled more by a mania secandi than by a knowledge of the pathology of the affection. Later the head of the femur was exposed and the capsule opened, the limb pulled down and the capsule folded over it in the expectation of the formation of a projecting shelf which would prevent the relapse of the reduced head. Subsequently, in the belief that no adequate acetabulum existed, after the incision for excision of the hip was made and the head of the femur turned out, attempts were made to gouge out an artificial acetabulum into which the reduced head could be placed. These methods naturally served simply as stages in

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the development of the treatment of the affection leading to more satisfactory undertakings.

When it was determined that in ordinary cases an adequate acetabulum is present with but little deformity of the bone, and that the chief resistance to reduction was furnished by the capsule and certain groups of muscles, a satisfactory method of operation by incision became possible. The operation is not a dangerous one if performed with care, though requiring thorough attention to anatomical details. The capsule is opened by a dissection which avoids injuring the muscles. The constricted and shortened portions of the capsule are divided, the head reduced, and the capsule stitched about the head. Where distortion of the head or imperfections in the shape of the acetabulum exists, these can be overcome by operations upon the bone in many instances. If a marked twist of the femur is present, it can be overcome by osteotomy. Elongation of the limb necessary for reduction can be made less difficult by tenotomy of the adductor magnus tendon and in some cases by subcutaneous division of the ilio tibial band and fascia lata, and by division of the hamstrings in more resistant cases.

It can be claimed that the operative treatment of congenital dislocation of the hip has now been placed upon a sound basis with a satisfactory record of success. It is, however, an aphorism in surgery that unnecessary injury to all tissues should be avoided. It is possible to reduce a traumatic dislocation of the hip by the aid of dissection, but no surgeon would attempt to use a knife in treating this accident unless manipulation had failed. It is, therefore, not strange that attempts should have been made to reduce by manipulation congenitally dislocated hips. It is well known that this has been accomplished, and the manual reduction of congenitally dislocated hips will be regarded as one of the achievements in modern surgery.

The details of this method need not be alluded to here, as they have been sufficiently described and are well understood. The method, however, has its limitations in resistant cases. Where a great amount of force is necessary

in reduction, fractures have followed, injury to the nerves, rupture of the femoral artery, and death has been reported even in the experience of skilful operators. Where the amount of force needed to overcome the contracted tissues is slight, the femur can be used as a lever without risk, but where the tissues require the use of force greater than the strength of the bone, fracture must result. Under these circumstances, mechanical aids in reduction have been used with success.

The simplest is a wooden wedge placed behind the trochanter, which serves as a fulcrum for the various stretching manipulations which are employed. More powerful, but less available, have been various traction appliances designed to elongate the limb, than have relied chiefly on a pulling force acting chiefly on shortened muscles. The use, however, of a precise force, elongating the limb and overcoming the contracted muscles, is not the only demand made upon an efficient mechanical appliance, but a force in an abducting direction, stretching the shortened tissues to abduct the limb, is also needed; and in addition a downward force, which would stretch the shortened capsular ligament before the entering head, pushing the head down and forward into the socket. If a mechanism is given the surgeon which will enable him to exert force with precision and certainty in all these directions, such an appliance will be great aid in the correction of this deformity. The apparatus recently used at the Children's Hospital (Boston) appears to meet these requirements, and has proved its efficiency in seventeen cases in which it has been employed at the Children's Hospital. The oldest of these was thirteen years of age, and the greater number were of ages at which manual manipulation is difficult. In one of the cases, a girl of nine with double congenital dislocation, the apparatus made reduction possible after all other methods had failed. In the two other cases reduction was not accomplished by the use of the Bartlett machine. Both were muscular children of the age of thirteen, with three inches shortening, and it did not seem safe to employ the apparatus to the full extent of its power. In

one of these the hip was later cut down upon, and a deformity of the head and acetabulum was found, making reduction impossible. It is possible that the same difficulty exists in the other case. Where marked bone distortion is present this cannot be overcome by any appliance. The machine used at the Children's Hospital can, however, be relied upon to exert all the power needed to overcome all contracted tissues.

RESISTANCE OF THE MUSCLES IN REDUCING A CONGENITALLY DISLOCATED HIP.¹

E. H. BRADFORD, M.D., AND L. T. WILSON, M.D.

The resistance offered to successful reduction in congenitally dislocated hips by the capsule has been demonstrated. In addition to this, it is evident that resistance is also offered by other tissues, notably the adductor group of muscles. It has been shown that the muscles attached to the pelvis and inserted on the greater trochanter are not shortened and do not offer resistance to reduction, but that the muscles which are attached to the pelvis and inserted along the femur below the greater trochanter do offer such an obstacle. In addition to this, the hamstrings also prevent the proper elongation of the limb. These tissues do not present such an obstacle in the younger cases as to prevent proper reduction, but in the older and more resistant cases the obstacle is considerable, and it is evident that, for surgical progress in advancing the age of possible cure, it is desirable to investigate the amount of resistance offered by these tissues. As complete dissection in cases of congenital dislocation of the hip has been impossible, owing to the lack of pathological material, our investigations were necessarily made upon normal tissues, and it was to determine the nature of resistance of tissues other than the capsule, encountered in elongation of the limb, that observations were made at the Harvard Medical School with the aid and advice of Professor Dwight of the Anatomical Department.

The problem which was presented was to determine what checked the lengthening of the limb under a strong traction after the capsule had been divided. The observations were made upon two adult cadavera and the cadaver of an infant, and it was demonstrated that if certain tissues were divided and the capsule entirely separated from its attachments, that the limb could be elongated by the employment of moderate manual force from one to two inches. Besides the capsule it

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was found that resistance was offered by the fascia lata, especially the ilio tibial band, by all the adductor group, and by the hamstring muscles. The reflected head of the rectus and the psoas and iliacus may be considered to offer but slight resistance. Where the adductors, the hamstrings, and the fascia lata were divided, it was found that the resistance offered was inconsiderable. The hamstring group can easily be reached by the knife; moreover, this group of muscles can be eliminated in resisting elongation of the limb if the limb is flexed at the knee. It is manifest that the fascia lata offers a strong resisting tissue in attempts to elongate the limb. This tissue, however, is strongest on the outer side of the leg and especially in that thicker portion which is termed the ilio tibial band. On slight abduction of the limb, this offers less resistance in attempts to elongate the limb. It, however, can be divided by means of a superficial tenotomy passed under the skin and drawn across the outer side of the limb in the same way as the plantar fascia is divided in correcting deformities of the foot. It would appear, therefore, that these tissues offer but little difficulty in surgical attempts in elongation of the limb. The adductor group presents a large, strong mass of muscles, and any attempts at extensive myotomy of these muscles would be impracticable, as this would amount to almost an amputation of the thigh. On examination, however, it would appear that the greater part of this group is composed of straight-fibered muscles which can be stretched without great difficulty. This is particularly true of the adductor longus, which has but few resistant inter-muscular septa. The same is true of the adductor brevis and the adductor medius, while the quadratus femoris from its situation offers no resistance in attempts to elongate the limb. The adductor magnus, however, remains as a large and resistant muscle. The upper and middle fibers of this, however, pass into the upper part of the femur and do not resist attempts at downward pull of the limb. It was found that the lower insertion of the adductor magnus consists of a strong tendinous band, collecting the fibers that pass from the tuberosity of the ischium and the posterior fibers of the muscles. This

tendon is inserted at the tubercle at the lower end of the femur, there being no insertion on the femur of the adductor muscles for two inches above, the upper fibers passing obliquely, while the posterior and inner fibers pass directly and end in a tendon which presents a strong obstacle to any downward pull.

Fortunately this tendon is easily accessible to the knife. It was found that after this was divided the chief resistance of the adductor group to a downward pull appeared to be eliminated.

In making these observations the pelvis was secured firmly upon a table and traction was exerted upon the ankle by means of a strong cord, a dynamometer being inserted in the cord to register the amount of force used. The hip capsule was weakened and practically divided by an anterior skin incision and cross division of the front of the hip capsule. The ligaments of the knee were found not to yield in a straight traction pull on the ankle of 150 kilos, and can be disregarded in this connection. In the full term infant cadaver separation of the lower femoral epiphysis followed continued traction after the tendon of the adductor magnus were divided.

The conclusion from these observations was clear, viz., that the resistance to an elongation of the limb of two inches offered by the tissues of the lower extremity other than the capsule could be minimized by a few incisions, and these at a distance from the hip, a fact of surgical importance in correction of resistant dislocated hip joint.

MECHANISM FOR REDUCING CONGENITALLY DISLOCATED HIPS.¹

RALPH W. BARTLETT, ESQ.

THE NEED OF A BETTER METHOD.

Congenital hip children when ready to undergo an operation to cure their deformity are healthy and vigorous, able to stand on their legs, to walk, run, play, and even to dance. Their deformity does not necessarily result in their ever losing the use of their limbs; consequently, while the existence of a congenital hip justifies reasonable treatment to cure it, yet it would seem that there could be no sufficient justification for any treatment so drastic as to endanger life, health, or the future usefulness of the limb operated upon; that if the dislocated head of the femur could not be placed in the socket where it belongs without breaking a bone, without causing paralysis, or without doing great damage to unoffending tissues the operator would hardly feel justified in taking any chance that such injuries would not be lasting or become permanent, and it can probably be assumed that no surgeon who has ever operated upon a congenital hip would hesitate to assert that he had taken no chance which he was not justified in taking.

Yet the results of operations, as reported by the operators themselves, make grewsome reading. The record shows, among other things, few bona fide cures, as many varieties of transpositions of the dislocation as words can be found to describe (whether a patient receives any compensating benefit from a transposition is debatable), several deaths, and an appalling list of complications, such as fractures, epiphyseal separations, paralysis of the sciatic branches, gangrene of the thigh, and every conceivable degree of shock.

Any one who has witnessed two or more operations to reduce the dislocation can bear testimony to the fact that the operator has to exert considerable muscular effort to

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reduce an easy case, and that when a resistant hip is encountered, the operation, which demands calm surgical discretion, degenerates into a veritable wrestle between the surgeon and his assistants on the one side and the inanimate limb on the other, and the records show that the limb successfully resists being cured in the majority of these cases.

MECHANICAL POWER A NECESSITY.

The trouble lies in the fact that the whole leg being as much out of place as is the dislocated head of the femur, any operation to successfully reduce a congenital dislocation of the hip joint must necessarily partake somewhat of the character of tearing the patient limb from limb.

In order to do this scientifically, the operator must be able to apply the last ounce of force which he is justified in using, be equally able to stop short when that ounce has been applied, and at all times during the operation be in a position to carefully observe all that is being done. But without the aid of mechanical power the operator is doomed in resistant cases to frequently find himself in such a fix as to be powerless to prevent the record of unavoidable complications from getting one or more additional items.

USE OF MECHANICAL POWER NOT NEW.

The idea of using mechanical power is not new. It originated with the first surgeon who undertook to reduce a congenital hip dislocation and has been spasmodically resorted to by every practitioner from his day to the present time. But the weights, the screw devices, the windlasses, and such other appliances have prevented the limb from being moved about while the power was being applied, and power alone, without motion and timely direction, has invariably proved unsatisfactory. The wedge-shaped block under the greater trochanter is, unfortunately, an efficient means for working injury. The shaft of the femur being used as the power end of a lever, the neck and head become the weight end, epiphyseal separations and fractures are

always likely to result; while the unavoidable slipping of the body generally results in bringing the edge of the wedge against the sciatic nerve at some time during the operation, thus causing those frequent cases of paralysis which, although they are generally only temporary, had better be avoided.

FIVE ESSENTIALS OF THE MACHINE.

A mechanical apparatus to be properly adapted for use in reducing congenital hips ought:

First. To avoid the danger of fracturing any of the bones involved by imposing the strain upon the obstructing muscles and ligaments rather than upon the bones.

Second. To avoid causing paralysis by avoiding injuries to the sciatic nerve.

Third. To avoid injury to any parts other than those being operated upon by confining the application of force to the leg and hip under treatment.

Fourth. The machine must be capable of furnishing all the power and all the motions needed, so as to leave the operator, not only free to direct the application of the forces at his command, but also in fit condition to keep such watch over the patient as to insure against needless injuries from any cause; and

Fifth. The machine should be sufficiently simple in its construction to be operated by the surgeon without requiring the attendance of a mechanic.

CORRECT THEORY TO WORK UPON.

No reductions should be undertaken unless the patient is in vigorous health, and careful measurements show the femurs to be of the same length. Then, if the leg to be operated upon can be moved about at will and forcibly abducted to 90° if necessary, while under a traction pull sufficiently powerful (1) to put all retarding muscles and ligaments on the stretch, and (2) strong enough to have moved the head of the femur from contact with the pelvis, the operator will have the femur in a most advantageous position for pushing

the upper extremity thereof slightly forwards and downwards to the socket.

With the femur in an abducted position, with every retarding muscle and ligament on the stretch, and with the head removed from contact with the pelvis, if the operator can also have a powerful, easy-working appliance adjusted at the upper extremity of the femur, which he can bring into play so as to force the dislocated head in the desired direction, it is obvious that the reduction can be consummated without serious injury to any muscles or ligaments except those which are actually holding the deeply-buried head in a false position.

It is also obvious that the muscles and tissues which must be penetrated, being separated, as they are, by the lower and anterior portions of the capsular ligament, can be penetrated and the head forced between and through them more readily if they are kept taut by traction than would be possible if they are loose and allowed to intervene between the head of the femur and the acetabulum.

THE MACHINE.

A detailed description of the various parts of the machine and of their relations to each other would necessarily be technical and presumably tiresome to persons disposed to look upon a machine as a means to an end and not as an end in itself.

Consequently no detailed description of it will be undertaken at present, further than to briefly describe it in a general way and illustrate with some of the patent office drawings.

Mankind is adapted to ride astride and suffer no injury therefrom.

Consequently, if the patient is placed astride of a properly constructed saddle seat and held there by traction on both legs, the pelvis will be secured without danger of injury.

The framework necessary to hold the saddle seat also holds strong pins or pegs at each side of the body, which materially assist in firmly holding the pelvis.

From these pegs emanate powerful leg extension rods

adapted to give all the traction pull needed and so attached to the pegs as to permit of every motion of which the hip joint is susceptible.

These pegs, being located in close proximity to the upper extremity of the femur, are also utilized to carry powerful, easy-working appliances which, having first been properly adjusted, enable the operator to forcibly loosen the head of the femur from those muscles and ligaments which hold it in a false position and to guide it between and through the intervening muscles and tissues to the rudimentary acetabulum where it belongs.

(1.) *Traction.* — Abundant traction pull is provided in three ways, namely:

1st. By a ratchet windlass on the lower or outer end of the extension rod (Fig. I., W.).

2d. By a leverage force somewhat similar to the force employed when a nail is pulled out of a board with a claw hammer (Fig. I., second position).

3d. By a 2-inch eccentric operated by a detachable wrench whenever the surgeon thinks there is any occasion for using this otherwise latent power (Fig. II.).

(2.) *Motions.* — All the abducting, adducting, flexion, extension, and rotary motions of the hip joint are provided by a simple system of hinges and collars playing upon solid cylinders.

(3.) *Joint Setting Power.* — The power for pushing the end of the femur forwards and downwards is supplied by a second eccentric superimposed upon the first, also operated by a detachable wrench and directed by the combined use of the wrench and a handle attached to the collar that surrounds the eccentric. To this collar is also attached the appliance which, having been properly adjusted at the upper end of the femur before traction is applied, is in readiness for use when needed (see Figs. III. and IV., D).

This femoral-head deflector is used first to weaken the ligaments which hold the head in its false position, thereby causing these to be the first to yield to the traction.

As abduction is gradually increased, the deflector assumes

a position between the pelvis on one side and the greater trochanter on the other, and in this position performs the function of the wedge-shaped block used by Lorenz. But without any of the dangers incident to the use of the trochanteric wedge alone, because the deflector is so placed and so shaped that it is impossible to bring it into a position to bruise the sciatic nerve, or any other important organ, if any care whatever is exercised. Thus the danger of causing paralysis is avoided. The danger of causing a fracture or an epiphyseal separation is also obviated, for the reason that the shaft of the femur is not utilized as the power end of a lever, but, on the contrary, is held in position only by traction on the muscles which surround the femur.

Although there is no drawing to illustrate it, yet it is obvious that as abduction is increased, the smooth, rounded surface of the deflector supplements the saddle seat as a counter-pull against the traction emanating from the end of the extension rod, and that the pelvis is held between the saddle seat and the deflector without increasing the traction upon the other leg.

There is an important principle underlying the seemingly trite saying, "The child who pulls too hard always gets the short end of the wish-bone," that holds good in an operation to reduce a congenitally dislocated hip, just as it holds good in an effort to remove a postage stamp from an envelope: there are times when the stamp should be pulled from the paper and times when the paper should be pulled from the stamp. The eccentric cam motions, each controlled by a detachable wrench, place the operator in a position where he can pull the body and hold the leg or hold the body and pull the leg, — the upper cam operating on the body and the lower cam only on the leg. But nothing less than congenital hip wisdom, which is something different from anatomical knowledge, can be relied upon to wisely determine whether the leg or the body shall be pulled at any given instant during the operation. Absolute immunity from all unnecessary injury is the purpose for which this machine has been designed, but it is not automatic.

TESTS AND RESULTS.

The apparatus has already been fairly well tested. Last spring experiments were made with it upon the cadaver of an aged adult.

As it was not possible to get a body with a congenital dislocation of the hip, it was necessary to make the tests upon a normal hip joint. This put the apparatus at a disadvantage, for the reason that the machine especially designed to reduce dislocations in which the head is always above the socket was called upon to force the head from the socket to a position below and anterior thereto. In spite of this handicap, however, the desired result was accomplished without difficulty, although two strong medical students had previously failed in their attempt to dislocate this same identical joint with a tackle. Confirmatory tests upon the other hip, which had not been subjected to the tackle tests, gave similar results.

Other experiments, to prove that the strain was imposed chiefly upon the muscles and ligaments rather than upon the bones, were made. These being satisfactory to Dr. E. H. Bradford, the machine was taken to the Children's Hospital, and has been used in a score or more of operations by Dr. Bradford.

Among these cases there have been no deaths, no fractures of any name or nature, no cases of even temporary paralysis, and as for shock resultant upon the operation, the doctor's own words upon an occasion when he had visited a patient the day after an operation may be quoted. The doctor said: "She has no more symptoms of shock than as if she had taken ether to have her hair cut."

A few of the hips reduced with the aid of the machine have already had their plaster casts removed and X-rays have been taken. All these indicate as good anatomical joints as the defective condition of the rudimentary sockets permit. That is, the bones are in their proper relative positions to each other, and there is motion in the joint. Whether nature will do her share in permanently reclaiming these joints yet remains to be demonstrated.

These results certainly justify the claim that the machine treatment is open to older sufferers, is less dangerous than either the cutting or the bloodless operations heretofore practised, while the results attained are as satisfactory, and most important of all, that it has placed the operation where it belongs, namely, in the category of reasonably safe experiments to cure a deformity which, however unfortunate, cannot be said to necessarily result in ever rendering these victims of Fate's caprice helpless cripples.

DANGER.

The machine, however, is entitled to only its fair share of the credit. Should its latent forces be directed and controlled by any one lacking in anatomical knowledge of conditions as they exist in congenital dislocations of the hip joint the machine would undoubtedly prove itself capable of adding item after item to that record of unavoidable complications hereinbefore mentioned.

The degree of rotation of the femur at the time the bones are placed in a position of fixation seems to present the next important problem to be solved. If a better position in which to secure the femur, after the dislocation has been reduced, can be discovered, the attainment of final and complete cures will undoubtedly become more certain, and the period of after-treatment will be materially shortened.

EXPLANATION OF DRAWINGS.

PLATE XXVIa.

Only left side of machine is shown: T, extension rod; W, ratchet windlass; S, adjustable saddle seat; P, pin carrying eccentric cams; A, handle by which P is placed in proper position for the operation; D, femoral-head deflector; H, handle to guide D; C, solid cylinder, engages rod T; E, detachable wrench in position for operating upper eccentric cam.

FIGS. I. and II. The upper or superimposed eccentric is not shown in these figures, which are intended to illustrate only the three forms of traction: First position, Fig. 1, shows traction applied by ratchet windlass W; second position shows traction caused by abducting the rod T to T'; observe arc described by radius centering at the fixed point where the

tuberosity of the ischium comes in contact with the saddle seat. Fig. II. shows the 2" throw which can be applied to the extension rod T. Fig. III. shows the two eccentric cams with their collars, the lower collar carrying the solid cylinder C hung on a hinge to give flexion and super-extension motions; the upper collar (also shown in detail in Fig. IV.) carrying femoral-head deflector D and the handle H. Fig. III. also shows the adjustable saddle seats S and the handle A to the screw which moves the post P towards or from the patient; the base F which supports the sacrum and the detachable wrench E engaging upper eccentric cam which revolves about the pin P and supplies its irresistible power to the deflector D.

PLATE XXVIb.

A photograph of the parts of the machine.

ON THE OUTPUT OF AMMONIA IN THE COURSE OF
DIFFERENT FORMS OF INSANITY.¹

P. A. LEVENE AND L. B. STOOKEY.

*(Clinical Observations made by Dr. G. H. Kirby.)**(From the Pathological Institute of the New York State Hospital.)*

It is generally recognized that any alteration in the chemical composition of an organ leads to a deviation in its function, and often to disease. The manner in which a change in the composition of the tissues is brought about may be very different; it may be the result of a foreign substance entering the organ, or the result of the chemical reactions constantly occurring in tissues during life, known as metabolic. Thus, for instance, in poisoning, intoxication, and to some degree in infection the relation of the normal tissue constituents may remain unaltered, and the normal function of the tissues is disturbed only by the presence in them of the toxin or poison. In conditions like uremia, coma diabetica, on the contrary, the tissues may contain no foreign substance, no abnormal tissue constituents; only the proportion, the relation of the normal components may be disturbed. It is self-evident that diseases produced in these different manners are distinct in their character. This becomes especially conspicuous when the attention is directed to the process by which the disease is combated and normal function restored. The harmful effect of a toxin or poison can be abated when the foreign substance is removed from the organism or when it is neutralized. In a disease of the second order the removal of any one substance or the neutralization of any one substance cannot result in a restitution of the normal function. It is therefore very important to make a distinction between diseases produced by mere intoxication, and those produced by changes in the chemism of the tissues. It is true that a toxin or poison can be manufactured within the organism by one organ, and that under normal condition the substance is prevented from

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entering the general circulation. However, if through some morbid process the substance is not removed from the organ by normal channels, it enters the blood and then the tissues, causing a disease analogous to any other intoxication. Diseases of similar nature — like jaundice — are justly called autotoxic. However, there was in recent years a strong tendency to classify many diseases of the second group under the autotoxic, and, in fact, every disease of obscure nature was regarded as an autointoxication. This is especially true in regard to the majority of mental and nervous diseases. The evidence on which this view was based was superficial and unsatisfactory. The discussion of it cannot be undertaken in this place. We shall only remark that it seemed to us a fruitless task to study mental diseases from the view of autointoxication. On the other hand, it seemed desirable to establish the existence or absence of any general pathological conditions in course of insanity, and if present to establish the relation of those pathological conditions to the mental symptoms.

The chemical changes occurring in an organism during life are so numerous and so complex that it is practically impossible to follow all the deviations that are theoretically possible in any given disease. We therefore limited our investigations to one phase of that cycle, which is generally known as nitrogen metabolism — namely, to the ammonia elimination.

The question of ammonia metabolism has attracted in recent years the attention of both the clinician and the chemical pathologist.

Ammonia is one of the final decomposition products of proteid material. It is formed on cleavage of the proteid by mineral acids and by enzymes of the digestive glands, as well as by intracellular enzymes. It is therefore a normal tissue constituent, and its presence serves for two purposes: first, to neutralize the acidity of acid substances formed within the tissues or introduced there from without, and second, to aid the transformation of some other products of metabolism into urea.

The ammonia elimination in disease was the topic of numerous researches in recent years; thus, by the aid of the method of Nencki and Saleski, Winterberg analyzed human blood in various pathological conditions, and came to the following conclusions:

1. That normal human blood contains preformed ammonia in small quantities, about 0.9 mgr. in one hundred cubic centimeters.
2. The ammonia content of the blood in fever undergoes great variations. There is no definite relation between the height of fever and the ammonia figure in the blood.
3. The comatose condition in yellow atrophy of the liver may occur without the presence of excessive quantities of ammonia in the blood.
4. Uremia cannot be regarded as carbamic acid intoxication.
5. In coma diabetica the quantity of ammonia in the blood may be considerably increased.

Camerer demonstrated that in coma diabetica the absolute quantity of ammonia in the urine is increased above normal, and so is the proportion of nitrogen in form of ammonia to the total nitrogen. Camerer thought that an attack of coma diabetica could be predicted by the quantity of ammonia in the urine. Krainski made estimations of ammonia in the blood of epileptics, and thought that he found an increase. His experiments, however, were very few, and were obtained by a method which was later proven to be not absolutely reliable.

In connection with insanity the object of the study of ammonia elimination was to investigate whether or not there were forms of insanity in the course of which symptoms of deviation in the general metabolism could be detected, and whether or not these forms differed clinically from those in the course of which no changes of general health were observed. Ammonia was therefore considered not as standing in any causative relation to the symptoms of the disease, but as an indication of disturbed metabolism.

Two forms with a fairly distinct clinical course are dementia

præcox and manic depressive insanity, and our attention was therefore directed particularly to their study. These two forms offered also great interest, for the reason that in the course of them mental and motor activity presented great variations, and it seemed desirable to investigate the correlation between these changes of the conditions and the state of general health and general metabolism.

We have realized of course that the character of ammonia elimination in the course of insanity may depend upon many incidental conditions like the mental state of the patient, and especially upon the character of the diet. Regarding the diet, there are statements in the literature on the question chiefly by Camerer, that vegetable food in a measure changes the absolute quantity of ammonia elimination, but that its ratio to the total nitrogen eliminated by the urine remains within normal limits.

However, since in the course of insanity the state of equilibrium may be less stable than in health, we thought it desirable to repeat the experiments on the two varieties of our hospital food, one chiefly of vegetables, the other chiefly of animal character. Four cases were selected for the experiment. Two of them showed a variable ammonia elimination in the course of the disease; the other two, on the contrary, showed an even normal ammonia elimination on ordinary mixed diet. The period of each experiment lasted three days. The food during the vegetable period consisted chiefly of asparagus, carrots, lettuce, and cauliflower. During the animal period the food was principally beef, eggs, milk, and bread.

TABLE.

Number of Case.	Date.	Character of Diet.	N. as Free NH ₃ Per Cent.
XV.....	5/20	Animal.	4.7
	5/21	"	4.8
	5/22	"	4.4
	5/23	Vegetable.	5.1
	5/25	"	5.6
	5/26	"	3.5
XXIII.....	5/21	Animal.	4.6
	5/22	"	3.3
	5/23	Vegetable.	3.2
	5/25	"	3.7
	5/26	"	3.5
XXI.....	6/2	Animal.	5.5
	6/4	"	5.5
	6/6	Vegetable.	6.3
	6/8	"	5.6
XIII.....	6/2	Animal.	4.9
	6/4	"	4.5
	6/6	Vegetable.	5.1
	6/8	"	4.5

Our observations corroborate the statement of Camerer that vegetable diet does not change the normal ratio between the total nitrogen eliminated by the urine, and that eliminated in the form of ammonia.

Our observation on the forms of insanity mentioned indicate in a general way that in the course of dementia præcox the ammonia elimination is less variable than in manic depressive insanity, and that in course of dementia præcox the ammonia elimination generally remains within normal

limits, while in manic-depressive insanity the ratio of nitrogen eliminated in the form of ammonia to the total nitrogen at times rises considerably above normal. This rise in the ammonia elimination seemed most generally to coincide with the periods of high motor activity.

We wish, however, to emphasize that motor activity, as such, does not necessarily cause a high ammonia elimination. Thus in a few cases of toxic delirium with motor activity no excessive ammonia could be detected in the urine. Nor does the presence of increased quantity of ammonia in the urine always coincide with the condition of motor activity. Thus, for instance, in case XX. high ammonia elimination persisted after all mental symptoms disappeared. The patient, however, relapsed.

Thus, for the present, we refrain from making any general conclusions, and publish the results of our work for the following reasons:

1. To illustrate the significance which we ascribe to symptoms detected by chemical methods. Increased ammonia eliminations, like increased acetone, oxalic acid, or indican elimination, has to be regarded only as a symptom of abnormal condition of the metabolism, or of an abnormal state of some of the internal organs. The cause of the deviation should be sought after its existence is well established.
2. To demonstrate that it is not impossible that different forms of insanity might be distinguished by the character of metabolism in course of the disease.
3. In order to invite more research along similar lines, so that sufficient data may be accumulated from which general conclusions may be formed with justification.

In concluding the review of our cases we should like to mention one case (XXII.) offering some theoretical interest. It was a case of chronic morphinism and cocaine poisoning. It was already mentioned that Hoppe-Seyler and his students, especially Araki, demonstrated that poisoning with morphine and like drugs leads to the appearance of symptoms of so-called "oxygen-starvation." As a result of oxygen-starvation the intermediate products of metabolism fail to undergo

their full oxidation and the blood and tissues are, so to say, saturated with substances of acid nature. Under such conditions it is natural to expect an increased ammonia elimination. In the case under observation the ratio of nitrogen eliminated in form of ammonia to the total nitrogen actually was much above normal.

We also wish to add that as yet no relation between ammonia and other constituents of the urine has been observed. Besides ammonia the chlorides, phosphates, and indican were estimated.

EXPERIMENTAL PART.

Acidity, chlorides, phosphates were estimated by usual volumetric methods, and indican gravimetrically. Ammonia was estimated by means of vacuum distillation. During the last few years several methods for the estimation of ammonia were devised. They were all based upon the distillation of ammonia under diminished pressure at low temperature and the reception of the distillate in a standardized solution of a mineral acid. All the methods offered some inconvenience: First, they required special apparatus; second, the operation could not be interrupted to test for the end of the distillation. A certain time, therefore, had to be found empirically as sufficient to distil off all the ammonia. This seemed to us not very reliable. We therefore preferred to proceed in the following manner: Twenty-five or fifty cubic centimeters of urine, to which enough of a three per cent suspension of magnesium oxide to make the urine alkaline was added and distilled at a temperature not exceeding 45° C. and at as low pressure as could be obtained, generally about 30 mm., until all the ammonia was distilled off, the condensing distillate being tested from time to time. Ordinary fractional distillation flasks were used for the purpose.

When the distillation was completed the residue was brought to a certain volume, and a nitrogen estimation was made in an aliquot part of the solution. In another sample of the urine a total nitrogen estimation was made. The nitrogen in form of ammonia was calculated by differences.

Since the absolute amount of ammonia eliminated by the urine has only little significance, and since the ratio between the nitrogen eliminated in form of ammonia and the total nitrogen is of interest, our process did not offer much additional labor in comparison with other methods.

To test the method, two estimations were made on the same urine. This was done on a great number of urines, and only a few will be quoted here.

N/10 cc. H_2 SO_4 required	59.00	in control	58.80
N/10 cc. " "	68.20	" "	68.40
N/10 cc. " "	35.30	" "	35.00
N/10 cc. " "	12.25	" "	12.20
N/10 cc. " "	13.00	" "	13.10

As seen from the above figures the results were quite uniform.

Further, urea is the most unstable nitrogenous constituent of the urine, and it seemed desirable to test whether or not, under the conditions of the process, it was being decomposed.

A solution of urea, therefore, was distilled under the same conditions as the urines. A nitrogen estimation was made before and after the experiment. The results are as follows:

N/10 cc. H_2 SO_4 required before distillation .	17.30 cc.
N/10 cc. " " after " .	17.35 cc.

The distillate was of neutral reaction.

No decomposition took place. When the nitrogen in the form of ammonia did not exceed five per cent of total nitrogen, it was considered normal.

(We wish to express our best thanks to Dr. Campell, of the staff of Manhattan State Hospital, for his kind assistance in the clinical part of the work.)

No. of Case.	Diagnosis.	Date.	Vol. of Day's Urine. cc.	Sp. Gr.	Acidity as HCl. %.	Cl. as HCl. %.	P ₂ O ₅ gram.	Indican, gram.	Total Nitrogen, gram.	Free NH ₃ %.	Remarks.
I.	Dementia Præcox	1-8	1,450	1,010	0.23	...	1.11	0.04	8.30	5.1	
		1-27	1,300	1,015	0.12	...	2.04	0.11	11.21	3.6	
		1-28	1,510	1,016	0.09	0.41	2.20	0.21	13.46	2.2	
		1-30	1,520	1,020	0.06	0.50	2.21	0.13	15.21	2.6	
		1-31	1,575	1,017	0.12	0.41	2.67	0.16	19.92	5.0	
		2-1	16,00	1,018	0.11	0.44	2.62	0.09	19.53	4.3	
II.	Dementia Præcox	1-7	1,050	...	0.11	...	1.74	0.07	6.21	5.1	
		1-12	980	1,023	0.09	...	1.81	0.09	12.66	6.1	
		1-13	1,200	1,017	0.04	...	1.27	0.03	9.64	3.2	
		1-17	890	1,022	0.31	0.19	...	0.13	13.48	13.1	} Albumin present
		1-18	520	0.12	13.54	7.3	
		1-19	500	1,023	0.07	0.48	...	0.14	8.21	5.4	
		1-20	400	1,030	...	0.87	0.64	0.11	6.61	4.7	
		1-22	500	1,024	0.19	0.58	1.33	0.16	7.99	3.8	
		1-23	325	1,027	0.27	0.43	0.84	0.05	2.73	4.1	
		11-12	1,550	10.01	3.5	
		11-14	2,235	16.43	4.2	
IV.	Dementia Præcox	2-19	2,175	1,006	0.01	0.14	0.76	0.16	6.06	8.2	
		2-23	1,020	1,020	0.12	0.26	1.58	0.09	12.11	2.5	
		2-24	720	1,019	0.09	0.27	1.32	0.02	10.14	2.6	
		2-25	1,180	1,015	0.07	0.23	1.35	0.07	9.28	9.1	Spells of crying and laughing with unusual resistance
V.	Dementia Præcox	2-26	675	1,021	0.15	0.45	0.94	...	7.73	6.2	Exalted
		2-28	1,200	1,012	1.75	0.25	11.50	3.3	
VI.	Dementia Præcox	3-8	600	1,020	0.16	0.36	1.32	0.04	7.72	2.5	
VII.	Dementia Præcox	2-11	720	1,013	0.05	0.53	0.36	0.06	3.82	7.3	Agitated, showing fear
		2-12	630	1,014	0.12	0.31	0.82	...	5.38	4.1	Ceases to show fear
		2-14	1,050	1,017	0.11	0.31	1.49	0.14	14.21	5.0	
		2-21	800	1,016	0.15	0.30	1.17	0.14	7.97	3.3	
VIII.	Manic Depressive Insanity	2-26	1,800	1,011	0.07	0.31	1.44	0.11	12.07	9.9	Motor activity
IX.	Manic Depressive Insanity	4-15	1,680	1,014	0.02	0.49	1.09	...	10.39	8.6	
		4-16	1,570	1,011	0.03	0.33	1.25	...	8.33	9.7	
		4-17	1,400	1,010	0.04	0.27	1.54	0.22	8.23	10.0	
		4-24	1,420	1,012	0.04	0.21	1.27	...	8.66	12.9	
X.	Manic Depressive Insanity	2-11	850	1,012	0.09	0.46	0.71	...	5.17	6.3	} Exalted
		2-16	910	1,015	0.07	0.39	0.94	0.05	7.17	6.6	
		2-21	900	1,017	0.05	0.44	1.18	0.33	8.03	5.6	} Improving
		2-27	1,875	1,014	0.07	0.42	1.87	0.17	13.42	5.8	
XI.	Manic Depressive Insanity	3-4	1,075	1,016	0.13	0.31	1.23	0.18	8.67	9.8	
XII.	Manic Depressive Insanity	3-5	780	1,023	0.19	0.13	2.00	0.14	14.74	8.8	Retarded and perplexed
		4-26	980	1,018	0.16	0.39	1.76	...	5.63	11.0	

No. of Case.	Diagnosis.	Date.	Vol. of Day's Urine, cc.	Sp. Gr.	Acidity as HCl. %.	Cl. as HCl. %.	P. O ₂ grm.	Indican, grm.	Total Nitrogen, grm.	Free NH ₃ %.	Remarks.
XIII.	Manic Depressive Insanity	11-29	2,160	2.37	0.21	13.97	8.5	
		12-2	2,160	1.51	0.26	9.28	18.1	
		12-3	1,325	1.67	0.11	8.57	7.6	
		12-5	1,325	2.14	0.18	11.69	4.9	
		12-6	1,300	1.57	0.13	10.56	4.5	
		12-7	1,400	1.32	0.10	7.99	12.1	
		12-8	1,600	0.32	10.75	5.2	
		12-9	1,450	0.17	11.52	5.6	
		12-10	1,090	0.15	9.94	8.4	
		12-11	2,300	0.63	17.10	8.0	
XIV.	Manic Depressive Insanity	1-6	770	1.011	0.09	...	0.63	0.03	5.12	4.4	
		1-7	1,200	1.016	0.09	...	1.36	0.08	8.98	2.8	
		1-8	1,430	1.012	0.05	...	1.21	0.11	19.08	8.3	Found on floor
		1-9	900	1.011	0.05	...	0.91	0.05	6.23	14.4	Elated, productive, and restless
		1-11	1,260	1.010	0.03	...	1.19	0.17	7.93	7.0	
		1-12	570	1.018	0.18	...	1.44	0.05	5.97	3.0	
		1-13	1,190	1.018	0.04	...	1.58	0.07	10.64	4.1	
		3-12	775	1.011	0.07	0.37	10.63	5.0	
		3-13	270	1.016	0.07	0.62	...	0.14	7.14	6.3	
		3-18	820	1.012	0.03	0.46	0.43	0.08	3.05	8.8	Dazed, perplexed
XV.	Manic Depressive Insanity	3-20	810	1.011	0.07	0.37	1.16	0.02	8.19	3.0	
		3-21	1,220	1.010	0.08	0.31	1.16	0.25	7.34	3.9	Inactive, dull and dejected
		3-22	1,100	1.018	0.13	0.46	2.23	0.30	11.94	6.5	
		3-23	1,250	1.018	0.13	0.34	1.50	...	8.96	5.0	
		3-24	1,300	1.017	0.05	0.23	1.68	...	12.65	6.8	Jumped from bed in excitement
		3-25	1,400	1.019	0.06	0.34	1.02	...	10.19	6.1	
		3-26	1,230	1.017	0.06	0.34	1.89	...	3.88	8.5	Restless, talkative
		3-28	1,000	1.015	0.07	0.32	1.44	...	11.95	3.7	
		3-29	1,080	1.017	0.09	0.33	1.60	...	14.47	3.2	No independent activity
		3-30	1,300	1.011	2.05	...	13.09	2.3	
XVI.	Manic Depressive Insanity	2-26	740	1.023	0.20	0.40	1.51	0.06	11.35	3.4	
		3-15	1,425	1.012	0.05	0.33	0.42	0.13	13.63	5.5	
		3-31	1,750	1.012	0.03	0.52	1.68	...	10.83	2.8	
		5-19	760	1.034	12.44	4.7	
		5-20	380	1.032	7.93	4.8	
		5-21	750	1.028	17.38	4.4	
		5-22	450	1.032	8.90	5.1	
		5-23	1,370	1.018	10.24	5.6	
		5-25	1,525	1.013	11.45	3.5	
XVII.	Depressive Hallucinosi	3-11	720	1.005	0.21	0.15	1.94	0.08	12.31	26.0	Bile pigments and acids present
XVIII.	Acute Alcoholic Hallucinosi	4-19	480	1.016	0.23	0.41	3.38	9.4	
		4-22	380	1.022	0.21	0.57	2.78	10.8	Proteid present
XIX.	Delirium	2-12	450	1.026	0.11	0.11	0.18	0.10	9.29	2.9	
		2-14	600	1.025	0.17	0.20	0.63	0.14	12.72	3.0	

No. of Case.	Diagnosis.	Date.	Vol. of Day's Urine, cc.	Sp. Gr.	Acidity as HCl, %.	Cl. as HCl, %.	P ₂ O ₅ grm.	Indican, grm.	Total Nitrogen, grm.	Free NH ₃ , %.	Remarks.
XX.	Toxic Delirium	11-24	5.64	13.8	
		11-28	650	0.52	0.06	5.38	4.6	
		12-3	700	1.003	0.13	0.06	0.86	42.0	
		12-4	1,040	0.41	...	2.88	9.0	
		12-5	650	0.43	0.10	3.12	9.0	
		12-6	840	0.95	0.10	3.23	13.9	
		12-7	880	0.17	3.87	12.7	
		12-13	2,090	4.09	10.5	
		12-15	730	0.05	2.88	8.3	
		12-16	390	0.10	2.22	7.0	
		1-2	470	...	0.14	...	0.76	0.10	5.77	3.9	
		1-4	1,090	1.008	0.18	...	0.52	0.14	4.73	8.2	
		1-5	1,470	...	0.03	...	0.97	0.08	6.48	8.6	
		1-12	1,260	1.014	0.10	...	1.53	0.09	11.29	7.8	
		1-13	1,280	1.013	0.10	...	1.16	0.08	9.55	15.3	
		1-14	730	1.017	1.21	0.04	6.89	26.6	
		1-15	1,650	1.012	0.08	0.39	0.95	0.16	10.41	4.9	
		1-21	2,200	1.013	0.03	0.44	1.23	...	10.47	10.8	
		1-22	1,720	1.010	0.03	0.26	0.70	...	7.44	10.8	
		3-17	2,120	1.007	0.07	0.30	1.04	0.08	10.49	7.2	
		3-24	2,220	1.005	0.05	0.34	0.97	...	6.72	8.7	Immediately preceding discharge
XXI.	Delirium with Typhoid Condition	3-11	220	1.029	0.20	0.18	0.35	0.04	2.14	5.4	Trace of proteid present. Diagnosed later as "Dementia Præcox"
		3-12	760	1.034	0.25	0.19	1.22	0.14	17.95	10.5	
		3-22	1,700	1.015	0.11	0.35	1.02	...	8.23	12.8	
XXII.	Chronic Morphinism	3-4	300	1.028	...	0.38	0.85	0.05	3.59	8.0	Trace proteid present
		3-6	300	1.007	...	0.39	0.48	0.01	0.73	12.3	Delirious
XXIII.	General Paralysis (Tabetic form)	3-8	2,800	1.010	14.42	4.5	During recovery
		4-8	2,000	1.010	13.23	5.7	
		4-21	780	1.019	12.60	4.6	
		4-22	1,030	1.015	14.42	3.3	
		4-23	740	1.019	10.00	3.2	
		4-25	1,350	1.012	8.39	3.7	
		4-26	860	1.015	9.51	3.5	

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THE PASSAGE OF TUBERCLE BACILLI THROUGH THE NORMAL INTESTINAL WALL. (A PRELIMINARY REPORT.)*

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In the discussion of the question concerning tuberculous infection through food, some writers have placed great importance on the location of the oldest or primary lesion, holding that this should be found along the digestive tract if this tube had served as the portal of entry. At first sight this would appear reasonable, but numerous experiments have demonstrated the fallacy of the claim.

In animals infected by feeding I have repeatedly been struck by the extensive involvement of the lungs, when the intestines were either free of lesions, or only slightly affected.¹ An explanation of these observations had to be sought for.

As early as 1890 Dobroklonski² showed that in guinea-pigs the tubercle bacillus could penetrate the intestinal wall in the absence of any demonstrable lesion, and after short contact.

In 1895 the late Professor Nocard³ made the interesting observation that blood drawn from horses under the strictest precautions would often show contamination if taken at certain periods of digestion, while at other times no such contamination would occur. In seeking an explanation of this, two of his students, Desoubry and Porcher,⁴ showed that in dogs during digestion of fat large numbers of bacteria were carried through the intestinal wall and could be detected in the chyle by plate cultures. If the dogs were fed material free from fat very few or even no bacteria were found in the chyle.

Quite recently (1902) Nicolas and Descos⁵ have shown

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that tubercle bacilli will pass through the healthy intestine of dogs during digestion.

My own experiments on this point were done during the fall and winter of 1902.

Method. — Healthy dogs were selected and kept under observation for some time. A purge of castor oil was given, and the animal fasted for twenty-four hours afterward. A single dose of an emulsion made of equal parts of melted butter and warm water, containing a large number of tubercle bacilli, rubbed into a smooth paste, was then given by means of a stomach tube. Three and a half to four hours later the dog was killed, and as much chyle as possible collected, together with the mesenteric glands. With this material guinea-pigs were inoculated intraperitoneally. Microscopic examinations were made also. The entire intestine was then carefully examined after washing it out, and in two cases microscopic sections were made from several portions of the gut. In no instance could any lesion be detected.

Results. — The experiments were carried out on ten dogs, eight of which gave positive results. From these eight, twenty-four guinea-pigs were inoculated. Of these, twenty-one showed well-marked tuberculosis, one was lost, and two remained well, showing no lesions whatever when chloroformed four months after inoculation. The macroscopic diagnosis of the guinea-pigs was in every instance confirmed by microscopic examination of sections. In only three cases could tubercle bacilli be demonstrated under the microscope in the material from the dogs used for inoculation.

The two dogs which gave negative results were the first two experimented upon. The probable explanation of failure in them is that the culture of tubercle bacillus used was of human origin which had long been used for making tuberculin, and was known to be of low virulence. The culture used was of the one hundred and forty-second generation. After this a bovine culture was employed, the generations being the twenty-ninth to the thirty-third.

In the light of these experiments we are warranted in concluding that under certain conditions tubercle bacilli pass through the normal intestinal wall with great facility and rapidity. The most favorable condition for this to take place appears to be during the digestion of food made up largely of fat. When we remember that the chyle is carried directly into the blood stream through the thoracic duct, it is easy to understand how it is that infection through food may show itself first in the lungs, or at any rate, that the lesion in the lung may be as old as the intestinal lesion.

The claim that a food tuberculosis should show itself in a primary intestinal lesion is fallacious and misleading.

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THE OCCURRENCE OF THE COLON BACILLUS ON THE HANDS.*

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The transmission of typhoid fever to whole communities at a time by infected water and milk has been in the past so conspicuous a phenomenon as to obscure the insidious spread of this disease from person to person by diverse more direct routes. The outbreaks at Bondsville, Provincetown, and Millville, studied by Sedgwick¹ in 1892, were among the first in this country in which the latter factor was clearly recognized; but of late years similar evidence has rapidly accumulated. At Newport, R.I., in 1900, of some one hundred and twenty-seven cases, thirty-eight were attributed by the investigator to an infected well, and the remainder to personal filth,² a dozen of the latter being reported in detail. Rieder,³ in his investigation of the epidemic at Riga in the same year, recorded thirty-six distinct chains of transmission from person to person. Schüder⁴ tabulated certain hospital statistics which showed that of thirty-five thousand six hundred and forty-seven cases treated, eleven hundred and seventy-nine, or three and three-tenths per cent, had originated within the hospital among the attendants upon typhoid patients. Sedgwick and the writer,⁵ in discussing the cause of that residuum of typhoid fever which persists even in communities having absolutely unpolluted water supplies, emphasized the importance of individual transmission and concluded: "Sometimes the route is so direct that typhoid fever, under uncleanly conditions, becomes for all practical purposes a contagious disease." According to Notter⁶ in his review of its prevalence among the British troops in South Africa, "The evidence that enteric fever is communicable from person to person has been slowly but surely accumulating, and few now would deny this source of dissemination."

* Received for publication Oct. 29, 1903.

When typhoid fever spreads in such a manner, the course of infection may be more or less direct, and varies in each individual instance; but the slow succession of the cases, often separated by a two-weeks' interval, is very characteristic. Some article of food generally forms the last link in the chain; and in many cases evidence points to the direct passage of the specific microörganism from the infected person to such substances. The latter condition presupposes, however, the presence of germs normally occurring within the intestine upon the outer surface of the body, and it is of importance to see what experimental evidence we possess upon this head.

The literature of the bacteriology of the skin is a long and interesting one. The earliest observers who detected microörganisms on the surface of the body attributed to their action all manner of pathological conditions; but Bizzozero,⁷ in 1884, examined the skin of healthy individuals and showed that yeasts, bacteria, and *Leptothrix* forms were of almost universal occurrence. Bordoni-Uffreduzzi⁸ two years later demonstrated again the existence of semi-parasitic organisms, mainly cocci, normal to the skin, and Behrend,⁹ Michelson,¹⁰ and Bender¹¹ arrived at similar results. Fürbringer¹² in connection with his disinfection experiments studied the bacteria under the nails, recording particularly the *Staphylococcus pyogenes*, var. *aureus*; and Unna¹³ monographed at some length the "Flora dermatologica." Mittman¹⁴ found seventy-eight different species under the nails, including numerous cocci and *B. pyocyaneus*. Maggiora¹⁵ isolated twenty-nine forms from the skin of the foot, and Preindlsberger¹⁷ recorded eighty species on the hands. In this country Welch,¹⁸ followed by Robb and Ghriskey,¹⁹ clearly distinguished between bacteria present by chance on the surface of the skin and those, like certain white and yellow staphylococci of low virulence, normally present in its deepest layers. Halsted¹⁶ and Moore,²¹ record the presence of organisms in intimate contact with the epidermis in the dog and horse respectively.

It is not, however, the semi-parasitic inhabitants of the deeper layers of the skin which interest us in this connection,

but the foreign bacteria present upon its outer surface. None of the observers above quoted furnish any evidence to confirm the inference drawn from practical experience that intestinal organisms are frequently transferred to the exterior of the body. As far as I am aware, but one investigation directly upon this point has been reported, an extremely significant series of experiments carried out by Wigura²⁰ in 1895. This author examined the hands of forty persons in the various wards of a hospital, both for the number and the kinds of bacteria present. He reports total numbers ranging from one hundred and twenty to twenty-three thousand four hundred bacteria per square centimeter of skin. Streptococci and staphylococci were, of course, abundant. The tubercle bacillus was found twice in ten examinations of persons in the phthisical wards; and finally from the hands of nine attendants in the typhoid ward *B. coli* was isolated six times and *B. typhi* once.

In the light of what has been said above, these results acquire considerable significance; and it seems worth while to add confirmatory evidence furnished by some experiments carried out three years ago in the Biological Laboratories of the Massachusetts Institute of Technology and not hitherto reported. They relate only to the presence of a single organism, *B. coli*; but they cover a larger number of individuals than the investigation of Wigura and of average normal individuals, not hospital attendants. Furthermore the bacteriological methods for the isolation and determination of *B. coli* were probably more rigorous than those available in 1895.

The subjects of the experiments were students in the Biological Laboratories of the Massachusetts Institute of Technology, janitors and servants connected with that institution, and pupils in a public grammar school of the city of Boston, girls between the ages of eight and fifteen, belonging mainly to families in poor but not indigent circumstances. The method pursued was as follows: Cups of crockery, each containing about two hundred and fifty cubic centimeters of water and a lump of cotton wool, were sterilized in closed

vessels. At the moment of the experiment, the cups were placed before the subjects and the latter were asked to wash their hands in the sterile water, carefully rubbing both the palm and back of the hand with the cotton, draining all water back into the cup, and finally replacing the cotton in the liquid. The cups were then put again into closed vessels and carried back to the laboratory, and a sample of one hundred cubic centimeters of the water in each cup was taken for analysis. The process used for the isolation of the colon bacillus was the generally satisfactory one developed by the State Board of Health of Massachusetts which consists in (1) the enrichment of a one hundred cubic centimeter sample by incubation for twenty-four hours at thirty-seven degrees with the addition of ten per cent of a bouillon containing ten per cent dextrose, five per cent peptone, and one-quarter of a per cent of phenol; (2) a preliminary test of one cubic centimeter of the enriched liquid by addition to a dextrose fermentation tube; (3) if dextrose is fermented, the inoculation from the dextrose tube of a litmus-lactose-agar plate; (4) if red colonies appear, the testing of pure cultures on agar, in gelatine, nitrate solution, milk, dextrose broth, and peptone solution. The weak point in the process is the possibility that a few colon bacilli in the presence of a number of streptococci or other forms may be overgrown in the preliminary dextrose tube and lost. That this method, however, gives more accurate results than the direct use of the lactose plate has been clearly shown by numerous observers in the study of polluted waters. It seemed possible that the addition of a tenth of one per cent of phenol to the dextrose solution in the fermentation tube might be of advantage in preventing such an overgrowth, and I therefore tried this method parallel with the ordinary method with twenty-four samples. In no case was a positive result obtained with the phenol-dextrose tube and not with the ordinary dextrose broth.

The results obtained from the examination of the one hundred centimeters' samples of wash-water are shown in the following table:

Experiment.	Subjects of Experiment.	Date.	Number of Individuals.	Dextrose Fermentation, Positive.	Lactose-agar, Positive.	B. Coli Present.	Other Forms Present.
1..	Students, M.I.T.	4.12.'00	11	0	0	0	
2..	School children (specially selected individuals)	14.12.'00	12	5	4	3	Type C.
3..	School children (taken at random)	20.12.'00	12	6	2	2	
4..	Students and instructors, M.I.T.	31.12.'00	12	3	2	1	Type A.
5..	Students and instructors, M.I.T.	8.1.'01	12	5	2	1	Type C.
6..	School children (ungraded class)	10.1.'01	6	0	0	0	
7..	School children	10.1.'01	6	1	0	0	
8..	Janitors and servants, M.I.T.	15.1.'01	9	3	1	0	Type B.
9..	Janitors and servants, M.I.T.	18.1.'01	7	4	1	1	
10..	School children (5th grade)	25.2.'01	12	3	1	1	
11..	School children (4th grade)	5.3.'01	12	1	1	1	
	Totals		111	31	14	10	

In thirty-one cases out of one hundred and eleven, the mixed culture obtained from the incubated water produced gas in the dextrose solution, and in fourteen of the thirty-one cases red colonies were produced in the litmus-lactose-agar plates.

The pure agar streak cultures made from these red colonies showed at once four types of development which proved to be correlated with differences of reaction in other media. A layer of medium thickness, but still translucent, with scalloped edges, covering the greater part of the slant surface in twenty-four hours, later becoming thicker and creamy white, but not stringy to the needle, invariably proved

to be *B. coli*. The reactions which I have considered characteristic, beside the growth on agar, are as follows: The fermentation of dextrose broth with the abundant production of gas in twenty-four hours; the fermentation of lactose in the litmus-lactose-agar plate with the reddening of the plate in twenty-four hours; the coagulation of milk in twenty-four hours; the production of nitrites from nitrate solution in twenty-four hours; the production of indol in peptone solution in three days; the formation of round or oval white colonies in the gelatine shake culture, often forming gas bubbles and not liquefying the gelatine in seven days.

An equally abundant growth on agar, but of stringy glutinous texture, noted above as Type A, proved to belong to a capsule bacillus, somewhat resembling the *B. pneumoniae*, but fermenting lactose rapidly, coagulating milk at 37° C. in twenty-four hours.* The growth on gelatine resembled that of *B. coli*, and that on potato was at first of the "invisible" type and later brownish. The reactions in glucose broth, peptone solution, and litmus-lactose-agar were those typical for *B. coli*, but nitrates were not reduced. The third type, Type B, produced on agar a heavy brownish-white growth, of granular texture, covering the whole surface in twenty-four hours. This form liquefied gelatine, producing a cup-shaped depression with a flaky sediment in the stab-culture, and formed somewhat ameboid colonies on the agar-plate. It produced acid, but no gas, from both lactose and glucose and did not form indol in peptone solution. It differed from *B. cloacae* of Jordan in not reducing nitrates and in coagulating milk more rapidly. Finally, Type C was at once distinguished on the agar-streak by the development of small, isolated, thin, translucent colonies, dotted here and there over the surface of the agar, instead of a rich growth along the streak. These organisms proved to be streptococci not clearly distinguishable from the *Streptococcus pyogenes*. They grew much more rapidly at 37° C. than at the room temperature, and formed spherical whitish

* For the detailed study and description of these organisms the author is indebted to Miss M. P. Hunnewell, to whom he wishes to express his thanks.

colonies on gelatin and potato. They produced abundant acid, but no gas, from both dextrose and lactose; they failed to reduce nitrates or to form indol.

The results given were obtained as stated above, by enriching samples of one hundred cubic centimeters. In all the experiments parallel examinations were made of samples of one cubic centimeter of the original wash-water by direct inoculation into the dextrose fermentation tube. In no case was the colon bacillus found in these small quantities of the water. Twenty-eight of the one hundred and eleven cultures gave gas in the dextrose tubes and nine of these fermented lactose. Of the nine pure cultures obtained, one was lost, five belonged to the Type C described above, and the other three to Type B.

In conclusion, *Bacillus coli*, as defined above, was found upon the hands of ten of the one hundred and eleven persons examined. In experiment No. 2 those children whose hands appeared especially dirty were selected for examination, but in all the other cases the subjects were taken at random. Excluding experiment 2, four positive results were obtained from the hands of forty-eight children; in the case of the technological students, thirty-five individuals yielded two positive results; sixteen of the servants and janitors gave one positive result. It appears, then, that colon bacillus was normally present on the hands of between five and ten per cent of the persons examined; and this makes it easy to understand how readily the typhoid bacillus may be transmitted by the hands of those affected with the disease or of their non-professional attendants.

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PROTOZOA IN A CASE OF TROPICAL ULCER ("DELHI SORE").

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The clinical and anatomical features of this peculiar skin disease are fully described in any modern text-book of dermatology, so that it is not necessary to give any lengthy description of them here. In brief, the disease consists of single or multiple focal lesions of the skin characterized by the formation of elevated, indurated areas, which ulcerate and eventually cicatrize. They have considerable resemblance to certain forms of cutaneous tuberculosis and syphilis. The lesions occur usually on parts not protected by clothing. Histologically the lesions consist in infiltration of the corium and subcutaneous tissue by cells, together with hypertrophy, atrophy, and disappearance of the epidermis. The affection lasts for months, or for a year, or longer. It is endemic only in tropical and sub-tropical countries. It is generally believed to be of an infectious nature and is capable of transmission from one individual to another by inoculation, but apparently is not contagious in the usual meaning of that term. There is evidence that mosquitoes and other insects may be the carriers of the infectious agent. The infectious agent has been sought for by a considerable number of observers, but with only negative, or contrary, or inconclusive results. Fungi, bacteria, and protozoa have been described in the lesions. The French observers particularly have written much concerning the occurrence of micrococci in connection with the disease, but have not offered convincing proof of their significance. At the present time no micro-organism has been satisfactorily shown to be the causal agent in the disease.

From an examination of the literature on the subject it seems that the results of only three observers are worthy of serious consideration in this paper.

D. D. Cunningham¹ in 1885 published the results of his examination of a specimen of "Delhi boil" that had been placed in alcohol immediately after its removal from the patient in Delhi. The epidermis over the specimen was intact and there was no evidence of ulceration. He studied frozen sections of the specimen, stained in various ways, and found that the condition was one of extensive cellular infiltration of the corium and subcutaneous tissue together with atrophy of the epidermis in some places and hyperplasia in others. In sections which had been stained with gentian violet and then considerably decolorized with alcohol he found a large number of peculiar bodies which he describes as follows:

"They varied very considerably in size: The average diameters of a series of measured specimens were $12.6\ \mu$ by $8.8\ \mu$, the largest measuring $12.8\ \mu$ by $25.6\ \mu$, the smallest $6.4\ \mu$ by $6.4\ \mu$. Such minute specimens as the latter were, however, rare, and as a rule they were considerably larger than the lymphoid elements among which they were situated. Their form also varied greatly. In some cases they were circular, in others elliptical, in others irregularly lobate. Their contour was in the majority of instances smooth, but in some of a more or less tuberculate character. In some specimens a very delicate cell wall was clearly visible. In others it was wholly unrecognizable or only to be detected on careful and special scrutiny. The distinctness with which they appear in sections treated with gentian violet is due to the elective staining of the nucleoid bodies which they contain by the dye. The number of such bodies present in different cells varies extremely, — in some cases only a single great nucleoid mass is present, seemingly occupying almost the entire cell body, in others a few of very various sizes occur, and in still others a large number of minute and fairly equal sized ones were thickly scattered throughout the entire cell. The cytoplasm in the gentian violet specimens remains almost uncolored; in those in which

¹ On the Presence of Peculiar Parasitic Organisms in the Tissue of a Specimen of Delhi Boil. Scientific Memoirs by Medical Officers of the Army of India, Part 1, 1884, Calcutta, 1885.

fuchsin has likewise been employed it frequently shows a more or less pronounced red hue. The tuberculate appearance presented by some of the cells is due to the numbers and sizes of the nucleoid bodies present in them, which in association form a mulberry-like mass pressing upon the cell wall and molding it to the inequalities of its surface. Such tuberculate bodies on superficial examination present certain points of resemblance to the characteristic bodies in cases of actinomycosis. On closer examination, however, it is evident that they correspond structurally with the description given above and are not due to any radiate aggregation of filaments. In certain cases appearances apparently corresponding with the occurrence of processes of cell division are present, the bodies of the cells being strongly constricted so as to form two lobes connected by a narrow neck, or two distinct cells occurring, which, from their relations to one another and the character of their opposed surfaces, seem to have just arisen, due to completion of such a process. In many instances, too, a distribution of the cells in little groups separated from one another by comparatively wide areas of granulation tissue can be recognized and may possibly be indicative of the antecedent occurrence of processes of division. The individual cells in some cases are closely packed among the surrounding lymphoid elements; in a large number of instances, however, they appear to lie in a limited clear space. This appearance may possibly be an artificial one, arising as the result of shrinking during the course of preparation. The number of cells visible in individual sections and in different parts of the same section varies considerably. In some sections as many as 80 or even more may be visible at once in a single field under a power of 140 diameters. In others they are present in varying but smaller numbers, and in almost any entire fields may in certain places fail to show any at all.

"After very careful consideration of the features presented by all the various forms present in the tissues, I am inclined to regard them as representing various stages of some simple organism of Mycetozoic nature. In the most

recent systematic treatise dealing with the Mycetozoa or Myxomycetes — Zopf's 'Schleimpilze' — they are subdivided into Monadinæ and Eumycetozoa, and it is to the former group that the organism here dealt with appears to me probably to belong. The appearances presented by the various forms are, according to this view, to be regarded as corresponding to various stages of the development, and, especially, of the development of the Zoöcysts or Sporocysts, of some Monadinic organism. Comparing the characters of the various specimens with one another, we have apparently to deal with the development of parent plasmodia or amebæ, which multiply by division and in which sporoid bodies are gradually developed, the process terminating in some cases with the formation of one great spore, in others with that of a dense aggregate of smaller ones."

It will be obvious to a histologist, from the reading of Cunningham's description of the bodies, that the morphological evidence adduced in favor of their parasitic nature is not sufficient to overcome the objection that they are elements of the tissue or degeneration products. This objection has the greater weight in view of the fact that Cunningham's histological technique was crude and not adapted to permit an adequate examination of the tissue, for he states that he made his observations on frozen sections. The plates that accompany the paper do not show any more morphological detail than is described in the text. It seems possible, however, that Cunningham did see among the various bodies that he describes the large cells described below, and that these large cells were what he regarded as "parent plasmodia" containing small spores.

Gustav Riehl,¹ in a paper published in 1886, reported the result of his examination of a single case. He describes among the infiltrating cells of the lesion many large epithelioid cells containing in their cytoplasm many bodies which he regarded as micrococci, with capsules, frequently more than twenty in a single cell. He regarded the bodies described by Cunningham as degeneration products.

¹Zur Anatomie und Ätiologie der Orientbeule. Vierteljahrsschrift für Dermatologie und Syphilis, 1886, p. 805.

R. H. Firth,¹ in 1891, states in a short paper that he could confirm the findings of Cunningham. This paper is but little more than a declaration that the author had seen in the lesions of the disease the same bodies described by Cunningham, and it contains nothing more convincing of their parasitic nature than does the paper of Cunningham. He proposed for them the name "*Sporozoa furunculosa*."

The case of tropical ulcer which is reported in this paper entered the service of Dr. R. B. Greenough in the Out-Patient Department of the Massachusetts General Hospital, July 28, 1903. The patient was a female child, nine years of age, born in Armenia. She presented in the skin of the left cheek, near the mouth, a firm, circular, elevated area, about twelve millimeters in diameter, covered with a blackish scab. This lesion had made its first appearance before the child left Armenia, some two or three months before. Through consultation with Dr. Charles J. White of the Dermatological Department of the hospital, the diagnosis of Aleppo boil or tropical ulcer was made. The lesion was excised and curetted by Dr. Greenough, and the material thus obtained, consisting of a piece of grayish translucent tissue about ten millimeters in diameter, and two or three smaller pieces of similar tissue, was immediately given to the writer for examination. Smear preparations were made from this material by rubbing and squeezing pieces of it against the coverglass, so as to cause a deposit of the tissue juices thereon. The smears were then immediately fixed and afterwards stained in various ways. In these preparations peculiar bodies were more or less distinctly visible. They were most clearly seen and differentiated from cell detritus in preparations fixed in pure methyl alcohol, and afterwards stained with the Romanowsky staining fluid for blood films described by the writer in a paper published in a previous volume of this Journal. ("A Rapid Method for the Differential Staining of Blood Films and Malarial Parasites." *Journal of Medical Research*, Vol. VII., No. 1, January, 1902.) After fixation by the methyl

¹ Notes on the Appearance of Certain Sporozoon Bodies in the Protoplasm of an "Oriental Sore." *British Medical Journal*, Jan. 10, 1891, p. 60.

alcohol the staining fluid was immediately applied to the preparation without washing in water, or allowing it to dry, and the process of staining continued essentially as described for blood films and malarial parasites in the paper referred to above. The best results were obtained in a preparation fixed with methyl alcohol for only a few minutes.

In the thinner and better fixed and stained portions of smears prepared in this way the peculiar bodies present the following characteristics: They are generally round, sharply defined in outline, and two to four micromillimeters in diameter. A large part of their peripheral portions is stained a pale robin's egg blue, while their central portions are unstained or white. A very prominent feature is the presence in each of the bodies of a larger and a smaller lilac-colored mass. The larger mass is about one-fourth or one-third the size of the body, is of variable shape, but always forms a part of the rounded periphery of the body. The smaller mass in some instances is round, in others is rod-shaped, and in the latter case is of a deeper lilac color than the larger mass. It is usually situated near or at the blue-stained periphery of the body. The blue peripheral portions of the bodies are usually sharply defined from the central unstained portion and sometimes show small unstained areas. A few of the bodies are oval or elongate in form. This is thought to be due to distortion in making the preparation, because in thin sections of the tissue such forms are not apparent. In the thicker portions of the smears the central portion of the bodies is stained blue as well as the periphery.

These bodies are present in very large numbers in the smears, often occurring in aggregations associated with a large nucleus, thus suggesting that they have been contained in a large cell whose outlines have disappeared in the process of fixing and staining. That this is true is shown in the sections of the tissue described below. (See Pl. XXVII., Fig. 2, and Pl. XXVIII., Fig. 3.)

The general morphology of the bodies and the large numbers of them visible in a single field of the microscope, under a high magnifying power, are indicated in Pl.

XXVIII., Fig. 5; Pl. XXIX., Figs. 6, 7, 8, and 9; and Pl. XXX., Figs. 10, 13, 14, and 15.

The constant morphology and structure of these bodies, the differential staining of their parts, their great numbers, and their position in cells seem to justify the belief that they are microorganisms and that they are the infectious cause of the lesion. Assuming that they are microorganisms, it seems reasonable to regard them as protozoa, because of their morphology and staining peculiarities. As to their classification among the protozoa, I am unable to give a definite opinion. Their small size, their great number, their intracellular position, and their morphology suggest that they are microsporidia. Nothing, however, was observed that suggested the developmental or reproductive cycle so characteristic of that group. On the contrary, certain appearances are observed in a few microorganisms, usually of larger size, which suggest multiplication by fission, which is a mode of multiplication apparently unknown among the microsporidia. These appearances consist chiefly in increased size and length of the lilac-colored masses with constriction in their middle parts, and in the presence in a single microorganism of two of the larger or two of the smaller masses or two of each (Pl. XXX., Figs. 10, 11, 12, 13, 14, and 16). In two or three of these microorganisms a process is seen extending inward from the peripheral portion and tending to mark the body into two symmetrical halves (see Pl. XXX., Figs. 10 and 11). Assuming that the lilac-colored masses are of the nature of nuclei, this duplication of them may be regarded as the preliminary process of division of the microorganism into two individuals.

I propose as the generic and specific names for this parasite *Helcosoma tropicum*. The generic name is derived from ἑλκος, a sore.

I do not adopt the name *Sporozoa furunculosa* that Firth applied to the supposed protozoan described by Cunningham in this disease, because that was an ameba-like, spore-forming organism and was obviously different from the one here described.

Microscopical examination of paraffin sections of some of the material which had been fixed in Zenker's fluid gave the following results: The lesion consists essentially of a very extensive infiltration of the corium and papillæ by cells, accompanied by atrophy and disappearance of the epidermis of the part (see Pl. XXVII., Fig. 1). The infiltrating cells are plasma cells, various kinds of lymphoid cells, and large cells with single vesicular nuclei and a relatively large amount of cytoplasm in which are large numbers of the microorganisms. These large cells, over extensive areas, are very numerous and constitute the principal part of the infiltration (see Pl. XXVII., Fig. 2, and Pl. XXVIII., Fig. 3). They are regarded as proliferated endothelial cells. The microorganisms are generally closely packed together throughout the cytoplasm of these cells and occupy most of the available space between the nucleus and the cell membrane. They are almost exclusively in these cells. Many cells contain twenty or more microorganisms. Only in very thin sections, cut with the aid of the Minot-Blake microtome, can the morphology of the individual microorganisms be clearly made out. In these thin sections all the microorganisms appear to be of spherical form, the cortical or peripheral portions staining faintly with nuclear stains and the principal portion of the body remaining unstained, while the larger and smaller lilac-stained masses described in the smear preparations stain deeply with methylene blue and gentian violet. (Pl. XXVIII., Fig. 4, shows three of the microorganisms in focus in a thin section.)

In thicker sections the microorganisms may give to the large cells in which they are situated the appearance of containing numerous basic staining granules of about the size of ordinary pus cocci, each surrounded by a clear space. These granules are the larger nucleus-like masses of the microorganism. The appearances are such as to make it certain that the cells containing micrococci with capsules, described by Riehl in his case and referred to above, were these same cells (see Pl. XXVIII., Fig. 3). As has been pointed out before, it seems possible that these same cells were seen

by Cunningham and by Firth, and represent some of the supposed plasmodia in process of sporulation described by them.

A part of the material was also used for the inoculation of a rabbit by subcutaneous injection, and by the scarification of the skin and of the cornea. No pathogenic effect was noted in the animal.

A small amount of the material was placed in a small quantity of freshly drawn human blood and kept in the incubator for some days. No evidence of multiplication of the microorganisms was obtained.

I wish to acknowledge my obligations to Dr. Robert B. Greenough for affording me the opportunity of studying this case, and to Dr. Charles J. White for helping me in the examination of the literature of the disease.

The interest aroused in the subject of protozoa in disease, by the work of Dr. W. T. Councilman and his pupils, has greatly stimulated the study of this case.

(For the benefit of those who may wish to apply the method used to other cases of tropical ulcer and who have not access to the paper referred to on p. 476, the following directions for the preparation of the staining fluid and for its application to smear preparations from the lesions are given:

Preparation of the Staining Fluid. — Dissolve 0.5 grm. of sodium bicarbonate in 100 ccm. of distilled water, and add to it 1 grm. of methylene blue (Grübler). Steam the mixture in an ordinary steam sterilizer for one hour, counting the time after "steam is up." The heating should not be done in a pressure sterilizer, nor in a water bath, nor in any other way than as stated. When cool, pour the mixture into a large vessel and add to it, stirring or shaking meanwhile, 500 ccm. of a one to one thousand aqueous solution of eosin (Grübler, yellowish, water soluble). In the mixture thus formed a fine blackish precipitate will be visible in suspension, and on the surface a scum with yellowish metallic luster will have appeared. Filter the mixture, collect the precipitate on the filter paper and allow it to dry thereon without washing. When thoroughly dry, dissolve this precipitate in pure methyl alcohol in a proportion of 0.5 grm. to 100 ccm. of alcohol. This alcoholic solution is the staining fluid. It will keep indefinitely, as will also the dry precipitate. Precautions should be taken to prevent the alcohol from evaporating, for thus the solution may become too saturated and precipitates may form on the preparation in the process of staining. If the staining fluid deposits such precipitates, it should be filtered and a small quantity of methyl alcohol added to it.

Method of Applying the Staining Fluid. — Place the fresh cover-glass preparation in pure methyl alcohol and allow it to remain therein for two or three minutes. It is probably best that the preparation be allowed to dry in the air before placing it in alcohol. Next remove the preparation from the alcohol, grasp it with coverglass forceps, and, without permitting

it to dry, pour onto it as much of the staining fluid as the cover-glass will conveniently hold, and allow the fluid to remain one minute. Then add water to the staining fluid drop by drop until a delicate scum with iridescent metallic luster becomes visible on its surface. Avoid diluting the fluid more than enough to just cause this scum to appear. If the staining fluid has been properly prepared, this scum will form before the fluid has been diluted enough to be transparent. The diluted fluid is to remain on the preparation for three minutes. During this time the most important part of the staining is effected. After this the preparation is to be washed with water until the nuclei of cells in the better-spread portions of the preparation appear well differentiated under a low power of the microscope and until any red blood corpuscles present have a yellowish or pinkish color. This will probably require about a minute's washing. The washing in water is important, for it removes superfluous blue stain and brings out the differential staining of the elements in the preparation. Distilled water should be used, for tap water may spoil the staining. The quality of the staining and the progress of the differentiation can be easily judged by placing the preparation, film-side uppermost, on a slide and examining it with a Zeiss AA or similar objective. When the decolorization is judged sufficient, the preparation is to be thoroughly dried and mounted in balsam. Dried stain adherent to the upper side of the coverglass may be easily removed with alcohol. The nuclei of cells should have a blue or deep lilac color and red blood corpuscles a pink or orange color. The cytoplasm of polynuclear leucocytes should show lilac-colored granules and the cytoplasm of lymphocytes should have a robin's egg blue color, while the protozoa should have the color appearances described.)

DESCRIPTION OF THE PLATES.

(Photographs by Mr. L. S. Brown, Clinico-Pathological Laboratory, Massachusetts General Hospital.)

PLATE XXVII.

FIG. 1. A section of the lesion under a low magnifying power, showing the extensive cellular infiltration of the dermis and the atrophy of the epidermis.

FIG. 2. A section of the lesion, showing the general character of the infiltrating cells. The granular appearance of the large cells is due to the presence of the microorganisms within their cytoplasm. x 500 approx.

PLATE XXVIII.

FIG. 3. A section of the lesion, showing the large cells containing the microorganisms under a higher magnification than Fig 2. x 1,000 approx.

FIG. 4. A very thin section of the lesion cut on the Minot-Blake microtome, showing the morphology of three of the microorganisms. The indefinite mass in the center of the figure is made up largely of microorganisms which are out of focus. x 2,000 approx.

FIG. 5. Smear preparation from the lesion stained with Wright's Romanowsky blood-staining fluid. The ring-like bodies with white central portions and containing a larger and a smaller dark mass are the microorganisms. The dark masses in the bodies are stained a lilac color, while the peripheral portions of the bodies, in typical instances, are stained a pale robin's egg blue. The very dark masses are nuclei of cells of the lesion. $\times 1,500$ approx.

PLATE XXIX.

FIGS. 6, 7, 8, and 9. Smear preparations stained as in the case of Fig. 5 and showing essentially the same things. All $\times 1,500$ approx.

PLATE XXX.

FIGS. 10, 11, 12, 13, 14, 15, and 16. All are smear preparations stained as described for the preceding figures. All $\times 1,500$ approx.

FIGS. 10, 11, 12, 13, 14, and 16 show the elongation, constriction, and duplication of the lilac-stained, dark appearing bodies in the microorganisms described in the text.

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CONTENTS.

	PAGE
SCARLET FEVER. PROTOZOON-LIKE BODIES FOUND IN FOUR CASES. (With two plates.) <i>F. B. Mallory</i>	483
A STUDY OF ACTINOMYCES CULTIVATED FROM COMMERCIAL VACCINE VIRUS. <i>W. T. Howard, Jr.</i>	493
THE SHAPE OF MAMMALIAN RED BLOOD CORPUSCLES. <i>F. T. Lewis</i>	513

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SCARLET FEVER. PROTOZOON-LIKE BODIES FOUND IN FOUR CASES.^{1 2}

F. B. MALLORY, M.D.

(*From the Pathological Laboratory of the Boston City Hospital and the Sears Pathological Laboratory of the Harvard Medical School.*)

In the skin of a boy (1) who died forty-eight hours after the first appearance of the eruption of scarlet fever certain peculiar bodies were found in the protoplasm of the epithelial cells of the epidermis, between these cells, and free in the lymph vessels and spaces of the corium just beneath the epidermis. The great majority of these bodies vary in size from a little less than two to six or seven microns in diameter. They present a series of forms with definite morphology, which strongly suggests that they are the various developmental stages of a protozoon.

These bodies³ found in skin fixed in Zenker's fluid and stained with eosin and methylene blue can be divided for further consideration into two groups. The first group consists of round, oval, elongated, and lobulated bodies, which stain delicately but sharply with the methylene blue. They vary in size from about two to seven microns, but occasionally considerably larger forms (ten to twelve

¹ Read at the meeting of the Boston Society of Medical Sciences, Dec. 15, 1903. Received for publication Nov. 26, 1903.

² This study of the skin in scarlet fever with the resultant discovery of the peculiar bodies in it is the outcome of the interest taken at the present time by the pathological department of the Harvard Medical School in the subject of pathogenic protozoa in consequence of the investigation of small-pox by Dr. W. T. Councilman and the men associated with him in that work.

³ I am under great obligations to Dr. E. E. Tyzzer, assistant in the pathological department of the Harvard Medical School, for his very careful and accurate illustrations of the bodies shown on Plate XXXI. The microphotographs on Plate XXXII, were made in the Pathological Laboratory of the Massachusetts General Hospital by Mr. L. S. Brown from thin sections cut on the Minot-Blake microtome by Dr. J. H. Wright, to both of whom I wish here to express my indebtedness.

microns) occur. Most of the bodies seem to be composed of a close-meshed, finely granular reticulum, but in some of the bodies the reticulum is coarse meshed. Occasionally a body is found containing one or more granules which are larger and stain more deeply than the others. It is not unusual for some of these bodies, especially those of small size, to contain one or more small but distinct vacuoles.

These bodies are common between the epithelial cells in the lower layers of the epidermis, where they often appear in rows extending out from the corium. In this situation they often present an elongated form. They are also numerous in the protoplasm of the epithelial cells, lying in vacuoles usually in the end of the cell away from the corium, but sometimes in the end near it, or in the middle of the cell at the side of the nucleus. In all these situations within the cell they cause indentation of the nucleus. In the corium the bodies are found close to the epidermis in the most superficial lymph vessels and spaces. The coarsely reticulated bodies occur only within epithelial cells, and are limited chiefly to the stratum granulosum. They sometimes reach a diameter of ten to twelve microns. The frequently elongated and lobulated forms of the various bodies of all sizes, both in the epidermis and in the corium, strongly suggest fixation while in ameboid motion.

The most striking and significant feature of the second group of bodies is their radiate structure. Such bodies are found both in vacuoles in the protoplasm of epithelial cells and free in the lymph spaces and vessels of the corium just underneath the epidermis. In the latter situation they often occur in clumps of two to four. These radiate bodies vary from four to six microns in diameter. They are usually spherical in shape, but occasionally, presumably from pressure, are somewhat elongated. They contain a central round body around which are grouped on optical section from ten to eighteen narrow segments, which in some cases are united, but in others are sharply separated laterally from each other. Occasionally some of the segments are larger than the others, and in their staining reaction and form resemble closely the

small bodies already described. Sometimes all of the segments are seen as small free bodies which still surround the central body, or seem as if they had been fixed while moving away from it between the cells. The central body in the rosettes is at first large and stains a deep blue, but as the segments enlarge and become free, it contracts and undergoes some change, in consequence of which it stains intensely with eosin.

Three pieces of skin had been preserved from this case. No record had been kept of the regions from which they were taken, but it is probable that they were excised in the median line over the thorax and abdomen, and from an extremity, because those were the regions generally chosen. In sections from one of the pieces of skin the bodies are very numerous in places; that is, they are not uniformly distributed, but usually occur in clumps. In the second piece of skin they are present in small numbers, while in the third none can be found.

Careful study of the tongue and various internal organs of this case, as well as of several lymph nodes and a clot from the heart, failed to show any bodies resembling those in the skin.

The findings in this case naturally led to the study of such material as was available, but unfortunately this was small in amount. In one case (2) a piece of skin preserved in alcohol showed the bodies as numerous as in the case already described, but they stained very poorly. In a third case (3) they occurred occasionally in small clumps in the skin, and one rosette form or body was found in a superficial lymph vessel of the corium, and in a fourth case (4) they were found in small numbers only in the epidermis of the tongue. In six other cases, where death occurred early in the disease, no bodies were found. In five of these cases only slide preparations made shortly after the autopsies were available for study, while in the sixth case a piece of skin from the axilla had been preserved, but freshly stained sections yielded nothing. A number of cases in the desquamative stage of the disease were examined with negative result.

In addition to these autopsy cases, small bits of skin¹ [one small piece three by five millimeters cut, with the patient's permission, from each of four adults (one a physician in the hospital), all ill with light attacks of scarlet fever] were studied, but no bodies were found. Blood smears, nasal and lachrymal discharges, and swab preparations from the back of the throat were examined from several cases with negative results.

Autopsies on cases of scarlet fever dying in the early stage of the disease are rare, yet they must be obtained before it is possible to determine what is the distribution of these bodies in the skin; that is, over what parts of the body they are most numerous and most constant. All that can be said at present is that in two cases in the early stage of the disease they were numerous in certain parts of the skin, while in two other cases, one at an early stage and another at a late stage, they were few in number in the material available for examination.

The grouping of the bodies would suggest that they are distributed more or less focally, like the bodies in small-pox, but there are no focal macroscopic lesions, as in small-pox, to call attention to them. The scarlet fever bodies were never found in the cells of the coil or sebaceous glands, or in the cells of the hair follicles except near the epidermis. They were often noticeably more numerous in the papillæ of the corium and the overlying epidermis than elsewhere.

For the demonstration of these bodies the routine methods of fixation and staining employed in the pathological laboratory of the Boston City Hospital proved the most successful, namely, the staining of paraffin sections of Zenker-fixed material first in a strong solution of eosin and then in a dilute alkaline solution of methylene blue. This method gives the sharpest stain and the most marked contrast. Various other methods were tried, but gave inferior results, as they hardly stained the bodies at all and gave no contrast.

¹ I wish to express here my indebtedness to Dr. John H. McCollom, resident physician at the south or contagious department of the Boston City Hospital, and to his assistants, Drs. H. H. Smith, W. W. McKibben, and A. E. Steele, for their kindness in obtaining for me blood smears and other material for examination from scarlet fever cases.

The eosin-methylene blue method is as follows :

1. Fix in Zenker's fluid.
2. Stain paraffin sections in a five per cent aqueous solution of eosin (yellowish, soluble in water, Grübler) for thirty minutes. Sometimes it is advisable to heat in the paraffin bath for fifteen to twenty minutes.
3. Wash off in water.
4. Stain for ten to twenty minutes in the following solution diluted with water in the proportion of one part of the stock solution to four or five of water.

Methylene blue (Grübler)	1
Carbonate of potassium	1
Water	ad 100
5. Wash off in water.
6. Differentiate in ninety-five per cent alcohol until the eosin color returns in the section and the nuclei are sharp. Keep the section constantly in motion, in order to obtain an even differentiation, and control the results with the low power of the microscope.
7. Absolute alcohol, xylol, xylol balsam.

The bodies stain a pale clear blue, except that the centers and the outer ends of the segments of the rosettes stain a deep blue. They stand out in fairly well-marked contrast to the purplish nuclei, pale pink protoplasm, and deep pink connective tissue.

These bodies can be interpreted in various ways,—as artifacts, degenerations, or protozoa.

Against the possibility of their being artifacts, such, for example, as precipitates, is the fact that in the three pieces of skin saved from this case they were numerous in one, comparatively few in the second, and entirely absent in the third, as well as in the other tissues of the case, although all the material was fixed and preserved in one and the same jar. The bodies were as numerous in the sections of the skin stained immediately after the autopsy as in those prepared at the present time, five years later.

It is difficult to see how these bodies can be due to degeneration, because they occur not only in and between the epithelial cells of the epidermis, but also free in the superficial lymph vessels and spaces of the corium. Moreover, they were found a number of times in epithelial cells which were in mitosis. Their size, morphology, and staining

peculiarities differentiate them sharply from the few degenerating polynuclear leucocytes, lymphocytes, and epithelial cells present in the skin. They are also entirely different from certain hyaline-pink-staining granules, due to degeneration, occurring in the epithelial cells of the coil glands.

In favor of the view that they are protozoa may be urged the fact that we have here a very definite series of bodies of characteristic and distinct morphology, which corresponds more or less perfectly with the cycle of asexual development (schizogony) of the malarial parasites. So far as can be determined by comparing these bodies with the tertian malarial parasite under similar conditions of fixation and staining, they are about one-fourth to one-third larger. Assuming that these bodies are protozoa, a series of changes from the small bodies to the radiate bodies or rosettes, together with the re-formation of the small bodies from the segments of the rosettes can be followed with comparative ease.

If they are protozoa, they may be normal or occasional inhabitants of the skin, or have a causal relation to scarlet fever. The first view is scarcely possible, because, in all the work which has been done on the skin in the last twenty-five years, bodies as definite as those described here could not have been overlooked. Moreover, in the very thorough examination of the skin of fifty-four cases of small-pox, made in the pathological laboratory of the Harvard Medical School during the past year, where the same methods of fixing and staining are employed, no bodies resembling those described here were found.

With the exception of two varieties of trypanosoma, it is impossible at the present time to grow protozoa in pure cultures; therefore we cannot fulfil the same laws in proving that a certain protozoon is the cause of a disease, as in the case of a bacterium. Moreover, it is not so necessary, because protozoa have sufficient differences in morphology to distinguish one variety from another, whereas in regard to bacteria this is not true. Koch's laws have not been applied

in their entirety to any of the protozoa at present generally acknowledged as the cause of certain diseases.

In order to prove that a series of bodies are stages in the developmental cycle of a protozoon we are dependent on three things, namely, ameboid motion, definite and characteristic morphology of the various bodies found, and the demonstration that the bodies go through a progressive series of changes or developmental cycle resulting in increase in size followed by division and the re-formation of the small bodies from which the series started. In hardened tissues we are restricted largely to morphology and the developmental cycle, although ameboid motion is often suggested by the forms of some of the bodies in their fixed condition. On one of Koch's laws we must, of course, insist; namely, that a protozoon, regarded as the cause of a disease, shall be present at some stage or other in every case of that disease.

Inasmuch as further research may prove beyond doubt that these bodies are various stages in the growth of a protozoon which has a causal relation to scarlet fever, the name of *cyclaster scarlatinalis* is proposed for it, in consequence of the frequent wheel and star shapes of the rosettes, its most distinguishing characteristic.

The situation of these bodies would suggest that they spread through the superficial lymphatics of the corium and from there invade the epidermis. How they reach the lymphatics is one of the many questions left to be solved. One observation in regard to them is of interest: they seem to bear no immediate relation to the slight inflammatory lesions often present in the skin, especially around coil glands and hair follicles, or to the marked lesions of the tongue.

Even if these bodies are protozoa, it is manifestly impossible to say anything in regard to the classification of them until more is known in regard to their life cycle. The majority of the forms suggest the schizogony of the malarial parasites. Certain other forms, however, — namely, those classed as coarsely reticular, — do not seem to belong in this cycle. They may be stages in sporogony or simply be due to degeneration of some of the other bodies.

SUMMARY.

In four cases of scarlet fever certain bodies were found which in their morphology strongly suggest that they may be various stages in the developmental cycle of a protozoon. They occur in and between the epithelial cells of the epidermis and free in the superficial lymph vessels and spaces of the corium. The great majority of the bodies vary from two to seven microns in diameter, and stain delicately but sharply with methylene blue. They form a series of bodies, including the formation of definite rosettes with numerous segments, which are closely analogous to the series seen in the asexual development (schizogony) of the malarial parasites, but in addition there are certain coarsely reticulated forms which may represent stages in sporogony or be due to degeneration of the other forms.

In conclusion I wish to say that while I personally believe that these bodies are protozoa and have an etiological relation to scarlet fever, I am far from claiming that such a relation has been proved.

1. Autopsy number, B.C.H., 98.242. Cornelius Buckley, three years old. Entered the contagious department of the Boston City Hospital Nov. 8, 1898. Had been ill two days. Eruption appeared on day of entrance. Boy practically moribund when admitted; skin of body covered with brilliant rash; tonsils swollen, covered with slight membrane; cervical lymph nodes much enlarged; temperature on entrance, 104.8° F., later reached 105.8° F. Died November 10.

Clinical diagnosis. Scarlet fever.¹

Autopsy ten hours post mortem. Whole body more or less deeply colored by scarlatinal rash; over chest and abdomen large maculæ of bright red, and on shoulders and in the femoral region all the skin of a deep cranberry red color.

Anatomical diagnoses. Eruption of scarlet fever; acute tonsillitis, with ulceration; slight ulceration of tongue; acute general hyperplasia of lymph nodes.

Cultures of heart's blood and of internal organs negative.

2. Autopsy number, B.C.H., 97.96. Rubie West, two years old. Entered the hospital March 25, 1897, with a well-marked eruption which had

¹ The clinical diagnoses of these four cases are on the authority of Dr. John H. McCollom, resident physician at the south or contagious department of the Boston City Hospital.

appeared that day. Died March 27. Autopsy the same day. Over arms, chest, and back was a general diffuse hyperemia, in places showing small pin-head points. It was most marked over the back, where it was confluent.

Anatomical diagnoses. Remains of scarlatinal rash. Acute general hyperplasia of lymph nodes. Cloudy swelling of liver and kidneys.

Cultures. Liver and kidneys, streptococcus pyogenes.

3. Autopsy number, B.C.H., 97.396. Nellie Cullen, ten years old. Entered hospital Dec. 18, 1897. Eruption appeared the same day. Died December 27. Skin showed well-marked eruption at time of death.

Clinical diagnosis. Scarlet fever

Autopsy, December 29. Skin over knees, ankles, and behind both thighs bright red; subcutaneous ecchymoses; skin over back mottled bluish red.

Anatomical diagnoses. Suppurative otitis media (double); empyema of both antra of Highmore and of sphenoidal sinus; general acute hyperplasia of lymph nodes; pleurisy with effusion; fatty degeneration of heart, liver, and kidneys; acute broncho-pneumonia; acute diffuse nephritis.

Cultures. Negative, except for bacillus pyocyaneus and bacillus diphtheriæ in sphenoidal sinus.

4. Autopsy number, B.C.H., 97.290. Nora Creedon, four years old. Entered the hospital Aug. 3, 1897, with diphtheria; isolated at once, on account of history of exposure to scarlet fever, and because of suspicious appearance of tongue. Transferred to scarlet fever ward August 18, on account of appearance of fine punctate rash on hips and arms. Died August 20.

Clinical diagnosis. Diphtheria, scarlet fever.

Autopsy on day of death. Skin clear, papillæ of tongue very much enlarged.

Anatomical diagnoses. Post-pharyngeal abscess; acute splenic tumor; acute general hyperplasia of lymph nodes; atelectasis of lungs; fatty degeneration of heart.

Cultures. Heart's blood, streptococcus pyogenes. Lung, streptococcus pyogenes. Post-pharyngeal abscess, bacillus diphtheriæ and streptococcus pyogenes.

DESCRIPTION OF PLATES.

EXPLANATION OF FIGURES IN PLATE XXXI.

The drawings were made with the Abbe camera lucida; projection onto table. Zeiss apochromatic homogeneous immersion 2.0 mm., apert. 130, compensation ocular 6.

Figs. 1 and 2 show numerous large and small scarlet fever bodies (stained light blue) in and between the epithelial cells of the rete mucosum. In Fig. 1 is a large body in a lymph space of the corium just underneath the epidermis. Several of the bodies suggest fixation while in ameboid motion.

Figs. 3, 5, and 6 are coarsely reticulated forms which may be degenerated forms of the scarlet fever bodies, or stages in sporogony.

Figs. 4, 8, and 9 probably represent stages preceding the radiate bodies. In Fig. 9 the bodies lie in a lymph space. It shows also four small forms which have just got free from a rosette.

Figs. 7, 10, 11, 12, 13, 14, and 15 show different stages in the development of the radiate bodies.

Fig. 10 is the earliest stage: there is a distinct central body and a definite, regular arrangement of granules at the periphery. Figs. 7, 11, and 12 show a little later stage of development: 11 and 12 are optical sections, while 7 is a surface view. Moreover, in Fig. 7 the body lies free in a lymph space in the corium. The segments begin to show a certain amount of lateral separation from each other. Fig. 13 is a still later stage: the segments are increasing in size and are more or less free from each other, although most of them are still attached to the central body. In Fig. 14 the segments are all free and enlarging, although still grouped around the central body. In Fig. 15 the bodies are still grouped around the central body, which is free and stains deeply with eosin.

EXPLANATION OF FIGURES IN PLATE XXXII.

Fig. 11 is from a fatal case of malaria; magnification approximately 1,000 diameters. All the others are from scarlet fever; magnification approximately 1,500 diameters.

Fig. 1.—Granular bodies lying in vacuoles in the protoplasm of epithelial cells, one in one cell, several in the other (following segmentation).

Fig. 2.—A large granular body, and a rosette in an early stage of development, in epithelial cells.

Figs. 3, 4, 5, 6, and 7.—Rosettes showing slightly different stages of development. In Fig. 6 the rosette is in the corium. All the others are in the epidermis.

Figs. 8, 9, and 10.—Later stages of the rosettes, showing the segments beginning to enlarge and separate from the central body. In Figs. 9 and 10 the rosettes lie within epithelial cells which are in mitosis.

Fig. 11.—Three segmenting tertian malarial parasites in a blood vessel in the brain. Fixation and staining the same as that employed for the scarlet fever bodies; magnification one-third less.

Fig. 12.—Two rosettes in epithelial cells situated near each other.

Fig. 13.—Four rosettes are present: three lie in epithelial cells; the fourth (in the lower left corner) is situated in the corium.

A STUDY OF ACTINOMYCES CULTIVATED FROM COMMERCIAL VACCINE VIRUS.*

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Introduction. — In October, 1902, in the bacteriological examination of a sample of a certain commercial vaccine virus submitted to me by the Health Department of Cleveland, four colonies of actinomyces grew in a single agar Petri plate which had been inoculated with the contents of two tubes of glycerinated virus. The colonies were all alike, and secondary cultures on various media proved them to be the same organism. The same actinomyces was cultivated shortly after from a second lot of the same virus. During the winter and spring of 1903, in association with Dr. William H. Weir,¹ I made a routine bacteriological examination of five makes of vaccine virus, three of which are extensively used by physicians in Cleveland. As will be seen below, actinomyces were found with surprising frequency. It is my object in this paper to call attention to the occurrence of actinomyces in certain makes of commercial vaccine virus offered for sale in this country, and to describe the varieties found. Actinomyces have been previously described in cultures from bovine virus by Sabrazès and Jolly² (1898) in France and by Folli³ (1897) in Italy.

Frequency. — The frequency with which actinomyces occurred, and their numbers, varied greatly. It may be said, as a general statement, that they were present more frequently and in greater numbers in those viruses containing the greatest number of bacteria. In our first series they

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were present in four out of thirty-two cultures made from the virus of five producers, but they were limited to that of a single firm (Virus I. below), being found in all this virus examined (two ivory points and two glycerinated tubes). In the second series they were present in twenty of sixty-three cultures made from separate points and tubes of the same producers. They varied, as a rule, from one or two to four or six colonies per plate. In one plate, however, nineteen colonies were counted

Altogether, actinomyces were found in twenty-four of the ninety-five cultures from the virus of five producers.

Methods. — The samples of virus studied in the second series were bought in the open market through a reliable pharmacist and were received invariably in unbroken packages. The virus was kept in an ice-chest, and the packages were broken when the cultures were about to be made. Agar Petri plates were made from ivory points of non-glycerinated virus and glass points and tubes of glycerinated virus. All precautions were taken to guard against air contamination; and it may be said here that in the nine years over which my experience extends in Cleveland, in the several laboratories under my direction, in cultures made by my assistants, students, and myself in teaching, research, and at autopsies, air contamination with actinomyces has been observed but once. In a large number of plate cultures made during research by several other workers in the same rooms in which the vaccine investigations were carried on, actinomyces, though looked for, were never met with. In making the cultures, both points and tubes were grasped with sterile forceps, and tubes were broken with a second pair of sterile forceps, and were not allowed to touch anything, or to stand exposed to the air. It is evident, therefore, that air and other sources of accidental contamination can be excluded.

Classification. — For convenience of reference, the makes of virus will be designated by the numerals I., II., III., IV., and

V., the cultures of actinomyces isolated from various viruses will be indicated by small letters of the alphabet, *i.e.*, I. *a*, II. *c*, etc., while the varieties or species under which these various organisms fall, after careful study of their morphological and cultural characters, will be classed under capitals, A, B, C, etc.

Of the twenty-four cultures of actinomyces obtained from the ninety-five cultures made, five from Virus I. were so evidently identical that they could be classified at once. The nineteen remaining cultures were studied comparatively upon various media. At the same time cultures on the same media, of identical composition, were made for comparison of all the known actinomyces I could get. These were obtained from the Johns Hopkins University, through the courtesy of Dr. N. M. Harris, and from Kral's Laboratory. They included *Actin. hominis* Berestenew, *Actin. Riviere*, *Actin. asteroides*, *Actin. farcinicus* Nocard, *Actin. Gabretchewski*, *Actin. albus* Cohn, *Actin. albus* Berestenew, *Actin. albus* of Kral's Laboratory; *Actin. graminarium* Berestenew, *Actin. orangeus*, *Actin. cin. niger aromaticus*, *Actin. violaceus*, *Actin. bovis* (Johns Hopkins Laboratory), *Actin. hominis* obtained from Dr. J. H. Wright, of Boston, and *Actin. bovis* isolated by me during the spring from a cow with actinomycosis. I was not able to identify any of the actinomyces obtained from vaccine virus with any of these actinomyces, except in the case of *Actin. violaceus*. As will be seen below, many of the cultures of actinomyces obtained by me from vaccine virus proved on comparative study to be identical.

The seventeen cultures fall into the following groups:

A is composed of cultures I. *a*, I. *b*, I. *e*, II. *a*, II. *c*, III. *a*, IV. *b*.

B is composed of culture I. *c*.

C is composed of cultures I. *d* and II. *c*.

D is composed of culture II. *f*.

E is composed of cultures II. *d* and II. *e*.

F is composed of culture IV. *a*.

G is composed of culture IV. *c*.

H is composed of culture IV. *d*.

I is composed of culture V. *a*.

I am forcibly impressed with the difficulty of classifying and identifying apparently new with previously described species * of actinomyces. The task, excepting the chromogenic and certain pathogenic forms obtained from cattle and man having certain characteristic features, seems almost hopeless. All my cultures differ more or less markedly from cultures of the various saprophytic and pathogenic species above mentioned, studied in comparison, as well as from the descriptions of these and other species of which I could not obtain cultures. Species A almost surely, and species B probably, have been described before, by Sabrazès and Jolly² in vaccine virus and possibly were identical with the *Actinomyces* of Rullmann⁴ and *Actinomyces* C of Damann,⁵ as will be pointed out later. Species C is to be classed with *Actinomyces violaceus*. The remaining six, D, E, F, G, H, I, are apparently new. After having been under observation for from six to nine months their cultural and other characters as below described have remained unchanged. The descriptions are based upon the results of the study of a number of cultures upon the various media used.

Though I have called actinomyces D, E, F, G, H, and I

* There is some doubt whether "variety" or "species" should be used here. If the classification of Lehmann and Neumann (*Atlas and Principles of Bacteriology*, 1901) is adopted, actinomyces are species of genus *Actinomycetes* and the different strains are varieties. Under the usual classification, to which it seems best to adhere, the various members of the group are species of genus *Actinomyces*; therefore the term "species" is used in connection with the *Actinomyces* described in this paper. Petruschky (Kolle and Wassermann's *Handbuch der pathogenen Mikroorganismen*, 1903, ix and x Lieferung, S. 832 *et seq.*) makes four species of filamentous organisms, *Actinomyces*, *Streptothrix*, *Cladothrix*, and *Leptothrix*, classifying them as *Trichomycetes* under the family of *Hypomycetes*. According to him, the species "*Actinomyces*" is characterized by the property of developing ray circle forms (*Strahlenkranzformen*) in living tissue, while *Streptothrix* presents a rich, true-branching, undulating growth which fragments and forms conidia chains (spores). It has been shown, however, by Lubarsch, Abbott and Gildersleeve and others that tubercle bacilli and certain other acid-fast bacilli may form typical "ray circles" in living tissue. Further, under this rule, all of the non-pathogenic organisms, commonly classed as *Actinomyces*, would be *Streptothrices*.

I have discussed the use of the terms "*Actinomyces*" and "*Streptothrix*" in a previous issue of this Journal (Vol. ix, p. 301, May, 1903).

new species, it is to be understood that I regard this as provisional and subject to correction should further work of others and myself prove this to be necessary. The organisms will be kept under cultivation for further comparative study.

Species A (Cultures I. *a*, I. *b*, I. *c*, II. *a*, II. *b*, III. *a*, and IV. *b*).

Glycerine agar. — Petri plates, after two days at 37° C., or four days at room temperature, show fine pin-head-sized, slightly elevated, sometimes tough, firmly-adherent, grayish colonies. Magnified fifty times the superficial colonies are light, yellowish brown in color, granular, with spreading, branching, peripheral filaments. Deep colonies are yellowish brown in color, granular, and show a concentric arrangement, with shorter peripheral branching filaments which are dark and refractive. Later the colonies reach a diameter of from three to five millimeters. They show a central elevation which gradually tapers toward the periphery, producing a distinct concentric appearance. After a variable time, always within eight or ten days, in many colonies the gray color gives place to a chalky whiteness on the free surface, while the under surface develops a yellowish-brown hue. Old dry, chalky colonies become thin and flattened.

Streak cultures. — After two days at 37° C. there is a raised, wrinkled, light gray growth, tending to spread from the borders, which have serrated margins. The surface is very uneven, and at the margins of the growth, as well as in isolated colonies, there is often a well-marked concentric arrangement. By the fourth day the growth on the upper portion of the media is dry and chalky white. The chalky material is readily removed with the needle, but the underlying growth is firmly adherent to the media. In from six to ten days nearly the whole surface of the growth is chalky white. Separate colonies in the growth often have a concentric appearance and a central depression. After thirty days the growth covers the whole surface of the media and is chalky white throughout. The wrinkling observed in young cultures has disappeared. The under surface of the growth and the media are yellowish brown in color.

In stab cultures there is a fairly well marked growth of small, grayish-white, round colonies and feathery material, which does not tend to spread. No chalky appearance develops in the depth of the media.

The growth on plain and glucose agar is identical with that on glycerine agar. In lactose litmus agar a faint pink color is seen by the fourth day at 37° C. Well marked pink color is present in two weeks.

Anaerobic cultures give a fairly well marked growth.

Gelatine. — Petri plate and streak cultures at room temperature resemble those on agar. Liquefaction begins on the fifth day. In stab cultures there is a luxuriant, elevated, grayish-white surface, which growth extends along the stab. Liquefaction is rapid after the fifth day, and in two weeks one-third of the media is liquefied. Complete liquefaction takes place

slowly. The liquid is clear, preserves its original color, and contains an abundant grayish-white growth.

Coagulated blood serum (Lœffler's). — On streak cultures, after twenty-four hours at 37° C., or after several days at room temperature, there is a decided, somewhat raised, slightly wrinkled granular growth of grayish-white color. In cultures with isolated colonies the latter are raised, grayish white, and about the size of a pin's head. After two days at 37° C. the whole growth is usually chalky white. The growth tends to spread and soon covers nearly the whole surface of the media. The margins are irregular. The growth often shows transverse wrinkling and is firmly adherent. The under surface of the growth is dark brown. Under the low power of the microscope the colonies have dark granular centers and gray margins from which spreading, branching threads project. Liquefaction begins on the eighth and is complete by the twentieth day.

Potato. — The growth is the same on acid and alkaline potato. At 37° C. a slight growth occurs in twenty-four hours, while in forty-eight hours there are numbers of raised, sometimes isolated, sometimes fused, grayish-brown colonies, without chalky appearance. After four days there is an abundant growth of a light yellowish-brown hue. Isolated colonies are round, elevated, with distinct central depressions, but without chalkiness.

Later the growth is of a dull gray color, raised, dry, wrinkled, and lichenous in appearance. The potato is discolored dark brown or black.

Litmus milk. — After five days at 37° C. acid formation is distinguished and the milk becomes clarified. There is an abundant growth at the bottom of the tube, as well as through the clear fluid.

Bouillon, plain and glucose, acid and alkaline. — The growth is alike on all varieties of bouillon tried. There is but slight growth after twenty-four hours at 37° C., but a decided growth in seventy-two hours, composed of numerous round or flat colonies from a millet seed to a pea in size, and a small amount of feathery material. The borders of the large colonies have a feathery appearance. All the colonies are white and translucent and lie at the bottom of the tube. A felt-like surface growth was observed after twenty days.

In all the media, but especially in old cultures on solid media, there is a very strong, mouldy odor.

Species A cannot be identified with any of the known forms of *Actinomyces hominis* or *bovis*, or with any of the white actinomyces which could be obtained for comparative study. It, however, appears to be closely related to, if not identical with, the *Actinomyces* of Sabrazès and Jolly² described in 1898 as a new streptothrix isolated frequently from fresh vaccine virus. Their short description almost entirely tallies with the characters of this group. They describe the early colonies as white, flat, chalky, round

islands with concentric borders and a small central depression. The under surface of the growth was of a light yellowish-brown color. On solid media the growth formed a granular, rough, white powdery membrane (the powdery appearance was lacking on glycerine agar), with a strong, mouldy odor. Gelatine, coagulated blood serum, and white of egg were liquefied. On potato the growth was chalky, with brown discoloration of the potato. Milk was peptonized. In fluid media the growth was confined to the surface. The organism was a strict aerobe. Optimum temperature was 37° C., but growth occurred from 15 to 40° C.

Microscopically the organism presented a fine, wavy, tangled, ramified, non-segmented mycelium, supporting filaments with free extremities. The mycelium and spores stained by Gram and retained carbol-fuchsin after treating with twenty per cent sulphuric acid. The spores resisted heating fifteen minutes at 75° C. Pathogenesis was not tested. With the exception of its acid-resisting quality, and the restriction of its growth to the surface of the fluid media, it is practically identical with Species A.

It is not improbable that the streptothrix isolated by Folli³ in 1897 from vaccine virus, and classed by him as *Streptothrix alba* (Rossi-Doria), is the same organism.

An actinomyces similar in certain respects to Species A is that described by Rullmann⁴ as *Cladothrix odorifera*, obtained from soil, and thought by him to be the cause of the mouldy odor of earth.

Damann's⁵ *Streptothrix C* resembles variety A also in its chalky appearance and musty odor.

Species B (*I. c.*). (Nov. Spec.?)

This form grows very much like A on solid media, but differs from it in (*a*) growth on agar, on which the colonies are smaller, gray at first and then light yellow along the line of the streak, with, however, some chalkiness on the upper part of the media and along the borders of the growth. In old streak cultures the growth is elevated, ragged, wrinkled, and granular, densely adherent, dark gray at the top, light gray and chalky white at the margins, while the central portion of the growth moist and yellow. The media is turned a dark brown color.

In plate culture the colonies are large,—3 to 6 mm. in diameter,—circumscribed, elevated, conical, and by the seventh day show a central depression. The colonies have a distinct concentric arrangement, and soon become chalky. They are tough and moderately adherent. Magnified fifty diameters, the colonies are dark brown and homogeneous, except at their borders where they have a finely granular appearance.

(b) There is no liquefaction of blood serum, though there is of gelatine.

(c) Growth on potato, on which the growth is yellowish at first, then becomes irregular, warty, and mauve colored. The potato becomes black.

(d) Growth in milk. Acid is formed, but no other change is produced in the milk.

The growth on all media has a strong mouldy odor.

Species C (I. *d* and II. *c*). Identical with *Actin. violaceus*.

Though not quite identical, these two forms are enough alike to be classed together.

On glycerine, glucose, and plain agar they form a dense, thick, wrinkled, adherent membrane of a light yellow color and without chalkiness. They form large gray, moist, translucent, porcelain-like colonies, which are irregularly round, elevated, conical in shape, tough, and firmly adherent. There is no chalkiness in ten days. The colonies have a central lighter zone, which later becomes umbilicated and sometimes forms a rather deep pit. They reach 5 mm. in diameter. When magnified fifty diameters, both deep and superficial colonies are homogeneous and dark in the center. Short branching threads project from the margins. Acid is formed in lactose litmus agar in two weeks. In agar cultures the growth, as well as the media, at a variable time takes on a violet hue.

Both cultures liquefy gelatine. I. *d* slowly liquefies coagulated blood serum, while II. *c* does not. They grow alike in bouillon, producing a large amount of feathery material, without round colonies, at the bottom of the tube. The media is not discolored. II. *c* does not grow on potato, while I. *d* forms a yellow growth on alkaline potato and a creamy white growth, which finally becomes violet, on alkaline potato. The potato is eroded and turns a dark violet color. Both coagulate and clarify milk. Both are strict aerobes.

These two organisms in many respects resemble *Actinomyces violaceus*, with which they were studied in comparative cultures. The chief differences noted were in the growth in milk and the rapid liquefaction of blood serum. *Actinomyces violaceus* like II. *c* refused to grow on potato. Old cultures have a sour odor.

For the present, at least, I am inclined to class these two with *Actinomyces violaceus*.

Species D (II. f). (Nov. Spec. ?)

Glycerine agar.—Petri plates after forty-eight hours in the incubator show pale white or gray translucent pin-point to pin-head sized, loosely adherent colonies. The smaller colonies are often grouped about the larger, which have a tendency to spread. In four days some of the larger colonies become dry and chalky. Magnified fifty times the superficial colonies are grayish brown or black in color with homogeneous refractive centers. About the margins short branching threads project.

Slant cultures show after twenty-four hours at 37° C. a faint translucent growth. After three days there is a considerable growth of fine pin-point size, grayish-white colonies which spread over the surface and tend to fuse, forming a granular growth, which varies from gray to lemon yellow in color. After ten days the growth is rather dry, granular, slightly wrinkled, loosely adherent, and lemon yellow in color. Later the colonies at the top, bottom, and sides become dry and finally chalky in appearance. The under surface of the growth is light yellow in color. The media also becomes tinged with yellow.

Stab culture.—A slight gray growth occurs along the side of the stab.

Glucose agar, slant cultures.—After forty-eight hours at 37° C. small white colonies appear. Later in from five to six days these have spread, and there is a fused, rather thin, non-adherent, wrinkled lemon yellow growth, covering the whole surface of the media. The growth at the top of the media becomes dry and chalky, the remainder of the growth becomes a dirty yellowish color after twenty to thirty days. The under surface of the growth becomes dark brown at the top and light brownish yellow below. The media is not discolored. On lactose litmus agar the growth is thin and grayish white; a decided pink color appears in twelve days.

Gelatine.—There is a slow, grayish-white growth. In fifteen days half the media is liquefied. The fluid portion contains a slight pale feathery growth; no surface growth; no pigmentation. Slow liquefaction with slight growth occurs in stab cultures.

Coagulated blood serum (Löffler's).—After two days at 37° C. there is a fine granular, slightly wrinkled growth of pin-head sized colonies. At the top of the media the growth is chalky. Magnified fifty times, the colonies have a dark homogeneous center, with a gray granular periphery from which thick spreading branching filaments project. By the sixth day the growth is well marked, but thin and somewhat sunken in the media. Liquefaction begins on the tenth day and proceeds slowly.

Potato, alkaline and acid.—The growth is the same on both. After four days at 37° C. there is a slight raised yellowish-brown growth. Later the growth becomes thicker, but does not spread or become chalky. The potato is not discolored or eroded.

Bouillon, acid glucose.—The growth is slow. After seven days at 37° C. there is a slight, feathery gray growth at the bottom, without globular colonies. No surface growth develops in twenty days.

Alkaline glucose bouillon.—After seven days at 37° C. a slight, feathery gray growth is observed in the bottom of the tube, while on the surface

there is a thick, wrinkled, chalky, felt-like mass covering one-third of the surface. By the twelfth day this spreads over the whole surface and supports several large oil drops.

Litmus milk. — After five days at 37° C. there is no change. By the tenth day the milk is clear, the watery liquid being light pink in color. By the twentieth day there is a chalky growth and oil drops on the surface. Later the color of the media becomes brown.

The odor of old cultures is faint, but musty.

There is a slight growth in aerobic cultures.

Species E (II. *d* and II. *e*). (Nov. Spec.?)

Glycerine agar. — Petri plates kept at 37° C. for two days show pin-point to pin-head sized colonies, which are thin, moist, translucent, porcelain-like, non-adherent, usually circular, sometimes irregular in shape, pale, and almost colorless. Chalkiness does not appear in ten days. Magnified fifty diameters, all the colonies are grayish brown in color and granular, with spreading branching filaments.

In slant cultures at 37° C. along the streak in two days there is a slight grayish-white growth of small colonies. After four days there is a thin, spreading, moist, adherent, pale gray, translucent, almost colorless growth, without chalkiness. After ten days the growth becomes somewhat raised, tough, adherent, and most of the surface is of a chalky white color. Some days later the under surface of the growth and the media itself become light brown in color. In stab cultures there is a growth of fine pale colonies. The growth on glucose and plain agar is essentially like that on glycerine agar.

Lactose litmus agar develops a pink color in twenty days at 37° C. The growth resembles that on glycerine agar.

Gelatine. — After several days at room temperature there is a fine grayish-white growth. In twenty days the upper three-fourths of the media is liquefied without the production of pigment. The fluid contains grayish-white, feathery material and globular colonies from a pin's head to a split pea in size. There is a small surface growth with a central glistening oil drop.

Coagulated blood serum (Löffler's). — After two days at 37° C. there is an abundant raised, dirty gray, spreading growth with somewhat irregular margins. The separate colonies are small and round. After four days the upper fourth of the growth is chalky white. Magnified fifty diameters, the colonies have black, homogeneous centres, while from the margins there project large, coarse, branching, radiating filaments. By the tenth day the whole surface of the growth is chalky white. The growth is firmly adherent. Liquefaction is quite marked by the twentieth day.

Potato acid and alkaline. — Repeated inoculations of a number of tubes resulted in no growth.

Bouillon, acid and alkaline. — No growth occurred after repeated inoculations, though in one tube there was possibly an increase in the size of the bit of growth with which the tube was inoculated.

Litmus milk. — Coagulation and acid formation were noted on the tenth day and clarification by the sixteenth day. The fluid later became dark brown in color and contained a copious thick growth.

Old cultures have a strong odor of putrefaction. The organism is a strict aerobe.

Species F (IV. a). (Nov. Spec. ?)

Glycerine agar. Petri plate. — After forty-eight hours at 37° C. the colonies are round or oval, grayish white, moist, porcelain-like, non-adherent, and of the size of a pin's head. There is no chalkiness or other change after ten days. Magnified fifty times, the colonies are granular with dark centres and widely spreading peripheral branching filaments.

Slant culture. — In forty-eight hours at 37° C. there are a number of pin's-point to pin's-head size discrete and conglomerate colonies which are slightly raised, moist, and of a grayish-white, translucent appearance. After nine days the growth is luxuriant, wrinkled, grayish white in color, with serrated margins. The lower portion is moist, the upper dry and chalky. The growth is firmly adherent to the media.

Stab culture. — There is after some days a faint grayish growth along the track of the needle.

The growth on plain and glucose agar is like that on glycerine agar. The media is not discolored. The growth is strictly aerobic. There is marked acid production in lactose litmus agar in twenty days.

Gelatine. — At room temperature there is a slight grayish-white growth in five days, both on the surface and along the line of the stab, with beginning liquefaction. There is considerable liquefaction by the fourteenth day. The media becomes finally completely liquefied, without discoloration, but with growth in the fluid.

Coagulated blood serum (Loeffler's). — After twenty-four hours at 37° C. there are numerous pin-head size, moist, gray colonies. After four days the growth is raised, moist, gray, and somewhat chalky at the top. Liquefaction begins on the sixth and is complete by the twentieth day. The culture has a decided odor of putrefaction.

Potato, acid and alkaline. — No growth occurred after repeated inoculations.

Bouillon, acid glucose. — There is a slight growth in twenty-four hours at 37° C. After five days there is a slight stringy, feathery growth at the bottom of the tube. There are no large colonies and no surface growth. The growth increases but slightly with time, and even after twenty days there is only a thin, cloudy growth limited to the bottom of the tube. In alkaline glucose bouillon the growth is marked in seven days, there being a thick, gray, felt-like mass at the bottom and on the sides of the tube.

Milk. — Milk remains unchanged and there is no acid produced.

The cultures have a distinct sour, putrefactive odor.

The organism is a strict aerobe.

Species G (IV. c). (Nov. Spec.?)

Glycerine agar. Petri plates. — After forty-eight hours at 37° C. the colonies are circular, elevated, moist, glistening, tough, loosely adherent, the size of a pin's head. After four days some are from three to four millimeters in diameter and are usually flat, gray, translucent, and porcelain-like. Many are chalky, with a central depression. They resemble the colonies of A, but lack the characteristic concentric appearance, however. Viewed from the under surface, the colonies have a yellowish-brown color. When magnified fifty times, the deep colonies are dark brown or black in color, and homogeneous in appearance, except at the borders from which short branching filaments project. The superficial colonies are dark gray, granular, with long spreading, branching, peripheral filaments.

On slant cultures after twenty-four hours at 37° C. there is considerable grayish-white, thin, rather moist growth with a few separate colonies along the line of the streak. In three days the growth is elevated, gray, dry, and spreading, with slight chalkiness in places. By the fifth day the whole growth is chalky white. The under surface of the growth is yellowish brown. By the fifteenth day the media has a light yellowish-brown color.

In stab cultures there is a grayish-white growth.

Glucose agar. — After two days at 37° C. the growth is gray, wrinkled, and adherent. By the tenth day it covers the whole surface and is chalky white. The media is discolored brown.

On plain agar the growth is much like that on glycerine agar, but not as luxuriant.

Lactose litmus agar. — Acid production is evident on the sixth day, and by the twentieth the media is pink throughout.

Gelatine. — At room temperature the growth is slow, and gray in color. Liquefaction is complete by the twentieth day. The media is not pigmented. In the fluid there are a number of small, gray, pin-head sized colonies.

Potato, acid. — In four days there is a light growth, not elevated, and of a pale yellow color. After ten days the growth nearly surrounds the potato, which is of a green hue. The growth is wrinkled and chalky white throughout.

Potato, alkaline. — After four days there is a raised grayish-white growth, which after fourteen days is wrinkled and lichenous in appearance. The potato is eroded, and of a dark brown color.

Bouillon, alkaline glucose. — After seven days there is a thin, feathery, gray growth at the bottom of the media; no growth on the surface. By the twelfth day at 37° C. the feathery growth shows a large increase; there are no round colonies, but a small, wrinkled, felt-like growth on the surface.

In acid glucose bouillon the growth is like the above, except that the feathery material is finer and no surface growth occurs. The media was not discolored.

Litmus milk. — There is no change before the sixteenth day, when coagulation occurs. The coagulum occupies the lower half of the tube, and above this there is a clear fluid. The media is completely clarified

by the thirteenth day, leaving a clear, pink fluid, with a white growth at the bottom of the tube.

The organism grows poorly under anaerobic conditions.

Species H (IV. d). (Nov. Spec.?)

Glycerine agar. Petri plates. — After two days at 37° C. there are gray colonies, from a pin's point to a pin's head in size. The colonies are raised, circumscribed, porcelain-like, and firmly adherent, with no tendency to spread. Magnified fifty diameters all the colonies are dark, homogeneous, and sharply circumscribed, with short, branching, peripheral threads. On slant cultures at 37° C. there is a well-marked raised growth, yellowish white at the top and grayish white at the bottom. After a few days the separate colonies and the denser growth become chalky white, except at the bottom of the culture, where the light yellow color persists. The under surface of the growth and the media are light yellow in color. In stab cultures there is a slight gray growth.

Glucose agar. — After two days at 37° C. there is a raised, wrinkled, tough, rather dry, yellowish-gray growth, with slight chalkiness at the sides and top. By the tenth day the whole surface of the media is covered with a thick, wrinkled, grayish-white, chalky growth. The under surface of the growth and the media are of a deep yellowish-brown color.

There is an intense odor of putrefaction.

Lactose litmus agar is turned pink in twenty days.

Gelatine. — The growth on gelatine slant is gray in color. Liquefaction is complete in fifteen days. The fluid contains a grayish-white, feathery growth and irregular colonies.

Coagulated blood serum (Löffler's). — After two days at 37° C. there is an abundant raised, granular, wrinkled, tough growth of a grayish-yellow color, which in four days becomes chalky at the top, while still moist at its lower part. Microscopically the separate colonies are dark and granular with homogeneous centers, and short, branching peripheral threads. In ten days the growth is of a dirty gray color, adherent, and without chalky appearance, except at the top. There is no liquefaction. Liquefaction does not take place in twenty days, in which time the media is quite dry.

Potato, alkaline. — After four days at 37° C. there is a marked raised, wrinkled, canary yellow growth, which is chalky white at the extreme top. After ten days the yellow color is lost, and the growth is dry, wrinkled, grayish white, and lichenous in appearance. The potato is eroded, and discolored brown.

Acid potato. — The growth is similar to that on alkaline potato.

Bouillon, acid glucose. — After four days at 37° C. there are at the bottom of the tube numerous small pin-head, globular, grayish-white colonies, with no feathery and no surface growth. After ten days some of the colonies are as large as a pea; no surface growth. The media is not discolored.

In alkaline glucose bouillon the growth is similar to the above.

Milk. — There is no change at 37° C., except a marked growth at the bottom of the tube, until the tenth day, when the milk is somewhat paler. By the twenty-first day the milk is thick, loosely coagulated, and white in color.

At the thirtieth day the upper three-fourths of the milk is a clear, light brown fluid, containing a thick growth, while at the bottom of the tube there is a grayish-white coagulum.

Old cultures on solid media have a putrefactive odor.

Species I (V. α). (Nov. Spec.)

Plate cultures, on glycerine agar. — After forty-eight hours at 37° C. there are numerous, grayish-white colonies, the size of a pin's head, or larger. The colonies are usually moist and glistening. Some show central pitting surrounded by a chalky rim. Finally, the colonies may reach 2 or 3 mm. in diameter. They are firmly adherent. Magnified fifty diameters, the deep colonies are small, and composed of branching, radiating, interlacing black filaments. There are no clubs. Surface colonies are gray, granular, with spreading, branching colorless peripheral filaments.

Glycerine agar. Streak culture. — In twenty-four hours there is a considerable growth of large, round, grayish-white, elevated colonies. In forty-eight hours the colonies are fused into a grayish-white, luxuriant, moist growth, covering nearly the whole surface of the media. At the top of the culture there is a slight, chalky appearance. With age, from two to three weeks, the growth becomes dry, and of a dirty gray color. In stab cultures there is a slight growth of small gray colonies. There is no pigmentation.

Cultures on glucose and plain agar have the same appearances described above.

Gelatine. — There is, after three weeks, a slight gray growth, without liquefaction.

Coagulated blood serum (Löffler's). — In forty-eight hours at 37° C. there is a profuse, grayish-white, somewhat granular, spreading growth, with serrated margins. After five days there is some tendency to wrinkling, but the growth shows no other change. The isolated colonies at the margins of the growth are round, elevated, gray, and granular, without chalky appearance. The latter is, however, seen at the top of the growth, where the media is dry. After thirty days there is a thick, adherent, dark gray growth, but no liquefaction.

Potato, both acid and alkaline. — After a few days at 37° C. there is a slight elevated, wrinkled, pale yellow growth, which is chalky in places. The potato is not discolored.

Glucose bouillon, acid. — In two days there is a slight feathery grayish-white growth at the bottom of the tube. After five days there is a marked feathery growth in the lower half of the tube; there are few round colonies. Later (seven to twelve days) large, flat, gray colonies develop. There is never any surface growth. In alkaline glucose bouillon there are

numerous large grayish colonies the size of a pea, or larger, which lie at the bottom of the tube. There is no surface growth.

Litmus milk. — There is no change (incubator at 37° C.) before the tenth day, when there is slight bleaching, but no coagulation or clarification. There is a heavy growth at the bottom of the tube. In sixteen days the milk is clarified, without coagulation, and develops a faint pink color.

Old cultures have a mouldy odor.

Oxygen requirements. — Species A, B, D, F, G, H, I grow poorly anaerobically, while C and E are strict aerobes.

Temperature relations. — All nine species grow at room and body temperatures, but more rapidly at the latter.

Cultures of the following species were killed after exposure to a temperature of 75° C. for ten minutes, A, C, H; B, D, F, and G were killed in thirty minutes; while E and I resisted this temperature for thirty minutes.

Morphology. — All nine species show on microscopical examination a mass of tangled intertwining, true branching threads. In some radiation can be made out. In every species the protoplasm of the branches is directly continuous with that of the threads from which they spring. They all stain well with methylene blue, gentian violet, fuchsin, and by Gram's method. None are either acid or alcohol fast after staining with fuchsin.

The threads and branches often show larger and smaller granular, unstained areas or segments, marking off longer and shorter bacillary and coccal forms.

In some species, notably A, B, C, D, H, there are larger and smaller irregular, deeply-staining swellings in the course of both threads and branches. Peripheral pear-shaped swellings sometimes occur. The branches are apparently always lateral and vary markedly in number and length. They often taper at the extremities. Both threads and branches are often undulating, and in many preparations a distinct envelope can be discerned. Spores in chains and in clumps are numerous in old cultures and in aerial hyphæ. None of the species show special spore-bearing structures. The threads vary in diameter, but in most forms are .5 μ thick.

Some (A and H) are uniformly thicker, reaching $.8 \mu$ in diameter.

Pathogenesis. — Rabbits and guinea-pigs were inoculated with all the actinomyces isolated.

Rabbits. — Subcutaneous inoculations of new and old cultures were uniformly negative, neither local nor metastatic lesions being produced.

Intraperitoneal inoculations. — Rabbits inoculated with bouillon cultures of A (cultures I. *b*, II. *b*, III. *a*) B, C (II. *c*) and F (IV. *a*) died in from five to seven days, but always without lesions, except congestion, and cultures from the peritoneum, liver, and spleen, kidney's and heart's blood were sterile. Actinomyces were not found in sections of the organs. The animals apparently died of toxemia due to substances in the cultures, rather than from the effect of the organisms, which were evidently killed in the body. Rabbits inoculated with all the other cultures (I. *a*, I. *c*, I. *d*, II. *f* (D), II. *d*, and II. *e* (E), IV. *c* (G), IV. *d* (H), and V. *a* (J), remained healthy, and when killed six weeks after inoculation showed no lesions, and cultures were negative. Guinea-pigs inoculated intraperitoneally with cultures of I. *a* (A), I. *c* (B), II. *f* (D), IV. *c* (G), IV. *d* (H) died from three to eight days without macroscopical lesions, and with no microscopical lesions save congestion of the liver, lungs, and kidneys. None showed peritonitis, and cultures from the peritoneum, liver, kidneys, spleen, and heart's blood remained sterile in all except the animal inoculated with IV. *c* (G), from the heart's blood of which the organism inoculated was recovered in pure culture.

Guinea-pigs inoculated with E, F, and I remained healthy, and when killed six weeks after inoculation showed no lesions, and cultures from their organs remained sterile.

Several of the species described in this paper, therefore, produce poisonous substances which kill rabbits and guinea-pigs, but there is no evidence that they are capable of producing suppurative or granulomatous processes in these

animals. Large doses of bouillon cultures of *I. α* containing spores caused no lesions when injected into the subcutaneous tissue of the neck of a calf.

These experiments neither establish nor disprove the pathogenesis of these organisms for man. Ponfick, Israel, Croakshank, and others have shown the difficulty of producing actinomycosis in cattle by inoculating bovine cultures of actinomycoses, and the rarity with which cultures from typical bovine and human actinomyces cause lesions in laboratory animals is well known. On the other hand, several of the saprophytic species are capable of producing lesions in animals. *Actinomyces violaceus* of Rossi-Daria causes pseudo-tubercles in guinea-pigs, and *Actinomyces pluricolor* of Gasperini and *Actinomyces Gruberi* of Terni are pathogenic for guinea-pigs, while *Actinomyces Hoffmani* caused purulent inflammation and fibrous tissue formation in rabbits. Many of the actinomyces as obtained from atypical actinomycosis in man are markedly pathogenic for rabbits and guinea-pigs.

Therefore it is evident that the presence or absence of pathogenesis for laboratory animals is not a true index of their pathogenesis for man.

Modes of contamination of vaccine virus with actinomyces. — As actinomycosis of calves is uncommon, and calves used for the production of vaccine virus receive fairly rigid inspection, it is very improbable that actinomyces reach the virus from actinomycotic processes in the calf.

It is probable that calves from actinomycosis infected herds with ample opportunity for getting bovine actinomyces upon their bodies may reach vaccine establishments.

It is not improbable that actinomyces may be transplanted from the vaccinal lesions of one animal to those of another, and that when these organisms once contaminate the virus of a vaccine establishment, they may be kept going in this way from one lot of virus to another.

The water, utensils, and instruments used in the preparation and storage of the virus are also possible sources of contamination.

Their presence in vaccine virus, however, is readily accounted for when we consider that various species of actinomyces have been found in air, water, soil, and on hay and straw, whence they may readily reach the hide, where they may be retained, even after washing and ordinary attempts at disinfection.

The possibility of the transmission of actinomycosis by means of vaccine virus.—Are the actinomyces present in vaccine virus pathogenic for cattle and man?

They are apparently not for the calves from whom the virus is taken. The significance of this observation is largely lost when the rarity of actinomycosis of calves is considered. Judging from the widespread distribution of various species of actinomyces in hay, straw, water, soil, and air, actinomycosis of both cattle and man is a comparatively rare disease, and one against which there is an apparent relative immunity or lack of susceptibility in these species; or else the pathogenic species of actinomyces are rare and largely parasitic.

As stated in the first part of this paper, there are but two recorded observations of the presence of actinomyces in bovine vaccine virus. Their presence does not appear to have been recognized by Copeman in England, Rosenau in the United States, nor by the bacteriologists of the numerous German and French government vaccine establishments and the commercial establishments in this country.

The failure of previous observers to find actinomyces in bovine vaccine virus may be due to (*a*), their absence from the vaccine studied (most unlikely in the light of my experience), or (*b*), that most of this work has been done to gain an idea of the number of foreign organisms present, and especially the presence or absence of pyogenic cocci, rather than to obtain a complete analysis by bacteriological methods.

I have not been able to find an authentic recorded case of transmission of actinomycosis by vaccine virus; indeed, I am not aware of a medical writer who considers its possibility.

At first sight conditions are against its occurrence, for the disease is not common in young calves and the post-vaccinal suppurative infections do not present the clinical picture of typical actinomycosis. Bacteriological studies of suppurative infections after vaccination are probably rarely exhaustive, but usually embrace simply the recognition of pyogenic cocci and a search for *B. tetani*.

The facts at our disposal are, however, susceptible of quite a different interpretation. It is now well established that acute suppurative lesions of various organs (brain, meninges, lungs, etc.) occur, due to actinomyces,— the so-called atypical actinomycosis,— and that in these processes the reaction differs in no respect from that excited by the pyogenic cocci and other excitants of acute suppurations, but lacks the chronic granulomatous characters of typical actinomycosis. Rosenbach has described an erysipeloid affection of the skin characterized by intense itching, sharply defined redness, and slow spreading, caused by an actinomyces. It is also to be remembered that in some cases the post-vaccinal mixed infections are chronic and associated with widespread necrosis. When these points are considered in connection with our observation of the presence of various species of actinomycoses in vaccine virus and the scanty negative evidence concerning their absence in post-vaccinal infections, it seems well within the range of possibility or even probability that some, at least, of these processes are caused by actinomyces.

Careful investigation of such processes by bacteriological methods will, of course, settle the question, and it is my purpose to do so at the first opportunity.

It is unlikely that the vaccination wound may be the portal of entry for actinomyces setting up acute and chronic processes in distant organs.

SUMMARY.

Actinomyces have been found in bovine vaccine virus by three sets of workers,— Sabrazès and Jolly, Folli, and myself.

The vaccine virus of five vaccine establishments in the

United States examined by me during the period from October, 1902, to May, 1903, contained actinomycetes — twenty-four times in a total of ninety-five cultures.

In the course of this investigation nine different species of actinomycetes, six of which are apparently new, were found.

These organisms probably reach the virus from the air, water, soil, hay, straw and hide.

While unsupported by bacteriological investigations, or by experiments, it is not improbable that some of the post-vaccinal suppurative infections are caused by these organisms, and are cases of atypical actinomycosis.

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THE SHAPE OF MAMMALIAN RED BLOOD CORPUSCLES.

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The red corpuscles of mammals, apart from species allied to camels, are usually described as circular bi-concave discs. When turned on edge, their optical section has the outline of a dumb-bell. This description is found in the text-books of Böhm and Davidoff, Szymonowicz, Schäfer, Kölliker, and Stöhr. In addition to these discs, Max Schultze (1865) found a small and variable number of spheres, five to six microns in diameter, with granular or finely indented surfaces. He saw transitions between the discs and spheres. In spite of various precautions to maintain natural conditions, Schultze believed it possible that the spheres were formed after the blood had been drawn. Ranvier (1875) found spheres five microns in diameter in fresh blood. Böhm and Davidoff, and Stöhr also, refer to these spheres, but all agree that the bi-concave disc is the usual form.

Leeuwenhoek, who was one of the earliest students of blood, in 1719 described the red corpuscles as *globules* with an indenting sinus, like vesicles of water which had received the imprint of a finger. Leydig (1857) figured such cup-shaped corpuscles, but wrote no clear description of them. Ranvier (1875) applied a heated metal bar to the under side of a slide on which fresh blood had been placed. Over the bar the well-known effects of heat were visible. Along the periphery the corpuscles remained "normal" discs. Between these zones, certain corpuscles appeared as if perforated, but on rotation they were seen to be "cap-shaped." Ranvier figured both aspects of the cap-shaped corpuscles. Most of these are vacuolated spheres due to excessive heat, but others may be free from any malformation. Rindfleisch (1880) studied the developing red corpuscles in guinea-pig embryos. After losing their nuclei, he found that the corpuscles were cup or bell-shaped. These later become

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bi-concave discs, due to impact with other corpuscles. The process he compared with the flattening of river pebbles. With a variety of reagents, however, he found that cup-shaped corpuscles were obtained from adult human blood. Dekhuyzen (1899) described bell-shaped nucleated corpuscles in *Petromyzon*. He observed non-nucleated forms of similar shape in mammals, and considered them a transitory stage of great morphological interest. It is recorded that his assistant saw cup-shaped corpuscles in blood drawn from his finger. Howell (1890) felt convinced that "the bell shape which Rindfleisch ascribes to the corpuscles which have just lost their nuclei is a mistake. The red corpuscles, even of the circulation, as is well known, frequently take this shape when treated with reagents of any kind, or even without the addition of any liquid." Von Ebner (1902) writes of bell or cap-shaped corpuscles produced in warmed blood "by a



FIG. 1. — Human red blood corpuscles. On the left, normal forms, as seen in 1% osmic acid and in 0.65% salt solution. On the right, shrunken forms, as seen in osmic acid and in Zenker's fluid.

thickening of the border on only one side of the disc." The same explanation, together with vacuolization, is said to account for similar forms seen in fresh blood mixed with 0.75 per cent salt solution. Finally Weidenreich (1902) after an exhaustive study declares that bi-concave discs are abnormal structures due to the cooling and consequent concentration of drawn blood. The form of corpuscle found in circulating blood is bell or cup-shaped. Having repeated Weidenreich's experiments, I can confirm this conclusion.

Blood from the finger, immediately placed on a warm slide, shows cup-shaped corpuscles in active motion. As the slide cools, and the corpuscles come to rest, they have

become bi-concave discs of the conventional form, arranged in rouleaux. If fresh blood is placed directly in 0.65 per cent salt solution, which Weidenreich considered isotonic, cup-shaped corpuscles are more permanent. They are globular, hemispherical, or somewhat conical in form, with an excavation on their basal side. Among the deep cups there are shallow ones, and some saucer-shaped discs. On basal view the cups show bright, well-defined central areas suggesting perforation. Together with the cup-shaped corpuscles there are some spheres with more or less roughened surfaces. They are generally regarded as derived from other forms, and it is still unknown whether or not they occur within the body. The average dimensions of the cup-shaped corpuscles, as determined by Weidenreich, are as follows:

Greatest diameter . . .	7.0 μ .
Diameter of cavity . . .	3.0 μ .
Height of corpuscle . . .	4.0 μ .
Height of cavity . . .	2.5 μ .

In water the cavity of the red corpuscle becomes shallow, forming a bright lenticular spot at the periphery of the circular membrane. This finally disappears, and there is left a shadow, which is the collapsed outer layer of the corpuscle.

If the finger is pricked and a drop of one per cent osmic acid is placed over the puncture, blood may enter the reagent without contact with the air. Many cup-shaped corpuscles are preserved, and they are quite like those seen in the isotonic salt solution. They occasionally form short rouleaux, in which the top of one cup rests in the concavity of the next one. Such a group may exhibit progressive flattening of the cup to the saucer shape. There are many shrunken corpuscles in the osmic acid specimen. If the tops of the cups fall in, bi-concave discs are formed. These are often asymmetrical, due to lateral displacement or unequal depths of the depressions. The collapse of the corpuscle may give rise to as many forms as could be made from indenting a felt hat. There is often a median ridge, or a

three-parted elevation. The orifice of the cup is also distorted.

Zenker's fluid acts quite violently on drawn blood. In the cups and shrunken forms clear bright vacuoles of varying size make their appearance. This vacuolization is not found in tissues preserved with Zenker's fluid. In fresh material properly hardened, the corpuscles are almost exclusively cup-shaped, many, however, not appearing in profile. In pig embryos of twenty to twenty-five millimeters, and in rabbits of fifteen to twenty millimeters, non-nucleated cups, often shrunken, are found among the round nucleated forms. The appearance of the cups seems coincident with the loss of the nuclei. In sections of adult mammals cup-shaped corpuscles are the rule. Weidenreich saw them in most of the domestic animals, and in the monkey and porcupine. I have found them to be the characteristic shape in *Sciurus*, *Arctomys*, *Procyon*, *Mephitis*, *Mustela*, and *Didelphys*. In preserved mammalian blood the typical red corpuscle is cup-shaped. The bi-concave disc is but one of several forms of shrunken cups.

It may be thought that the depression which makes the cup is itself due to shrinkage, or to vacuole formation. The only proof to the contrary is to be had from the circulating blood of a living animal. In the mesentery of a rabbit Weidenreich saw that the corpuscles were like those in the preserved specimens, cup-shaped. An examination of the omentum of a guinea-pig has confirmed this observation. The flowing corpuscles were seen to be flexible bodies, somewhat variable in their proportions, some deeper, some flatter, but all that could be clearly observed were cup-shaped. We concur, therefore, in Weidenreich's general conclusion that "Die roten Blutkörperchen der Säugetiere die Form von Glocken haben."

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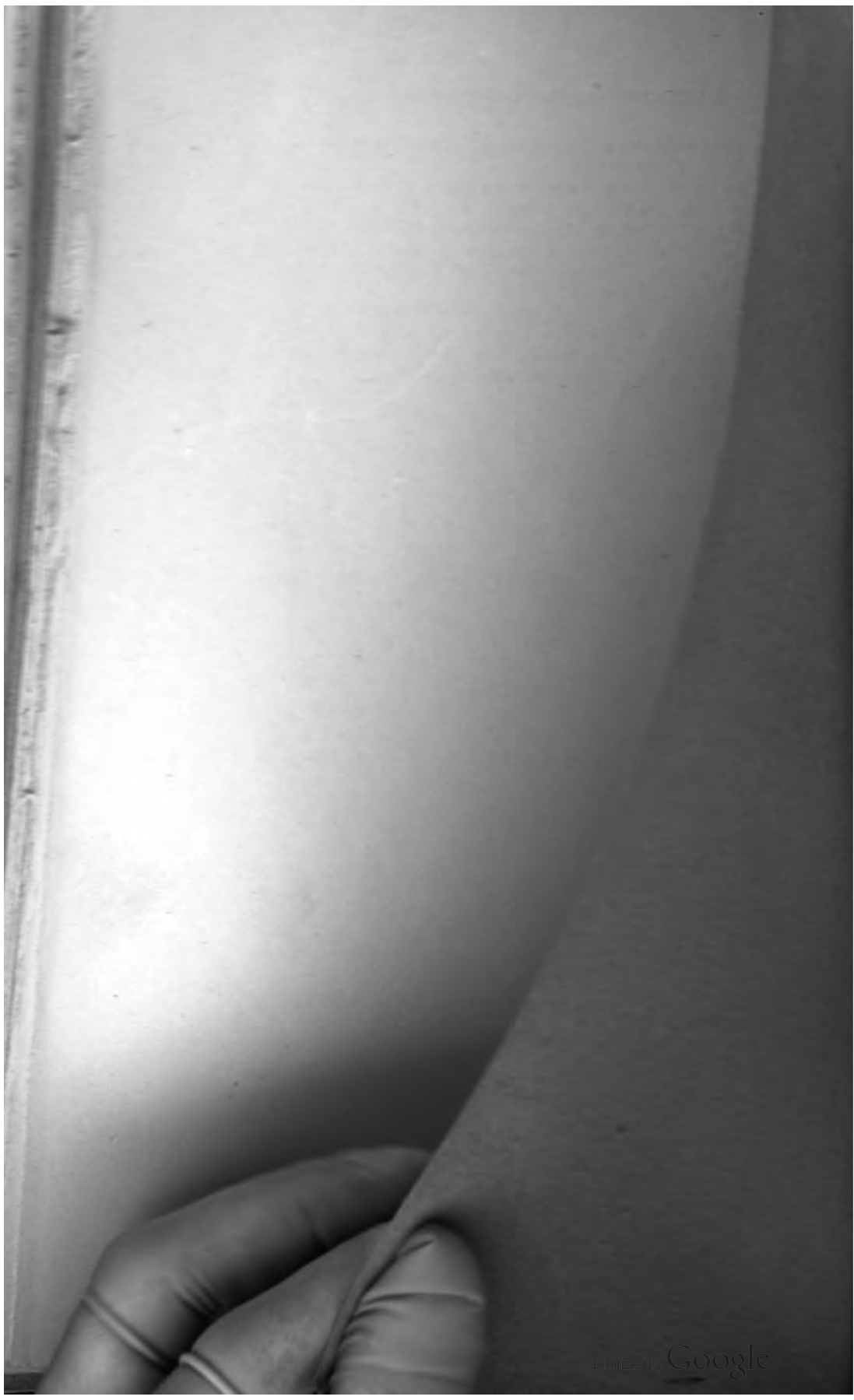
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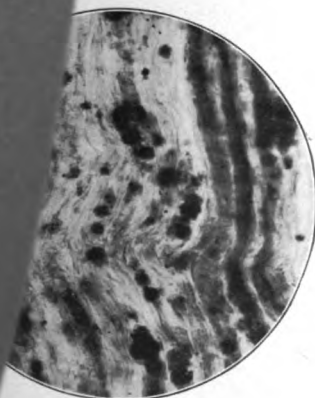
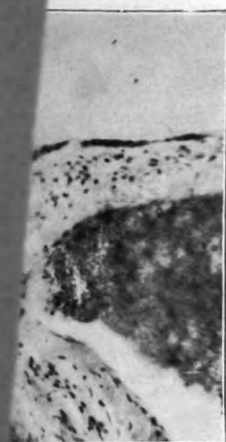


FIG. 3.

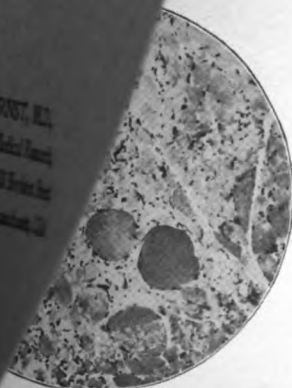


FIG. 4.

LEAD POISONING.

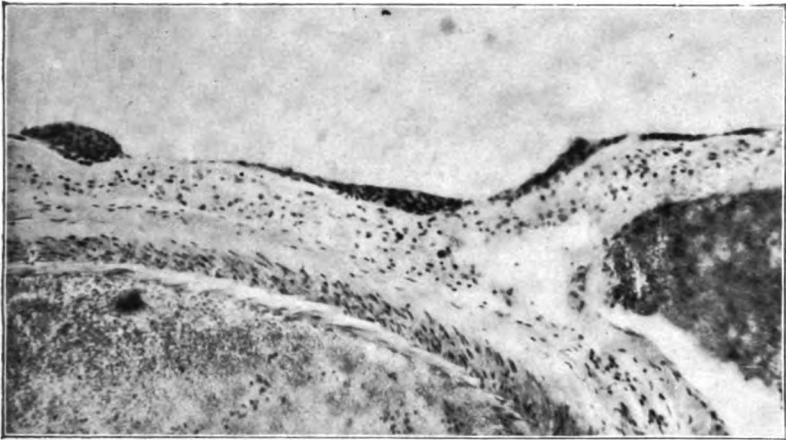


FIG. 1.

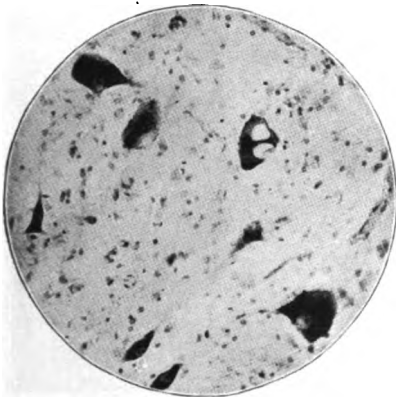


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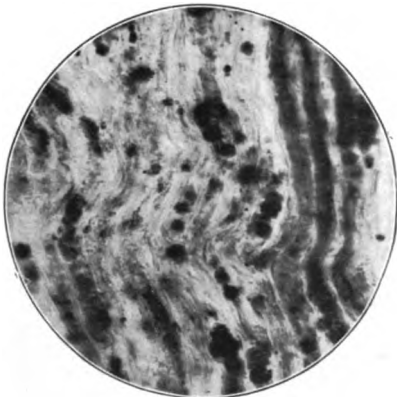


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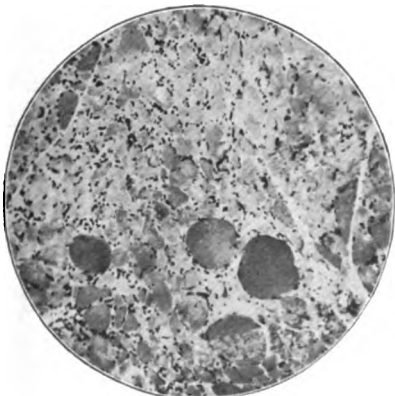


FIG. 4.

SPILLER.

LEAD POISONING.

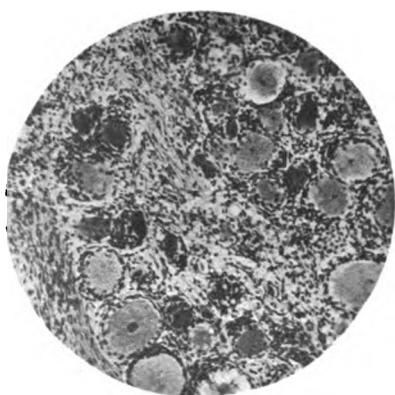


FIG. 1.



FIG. 2.



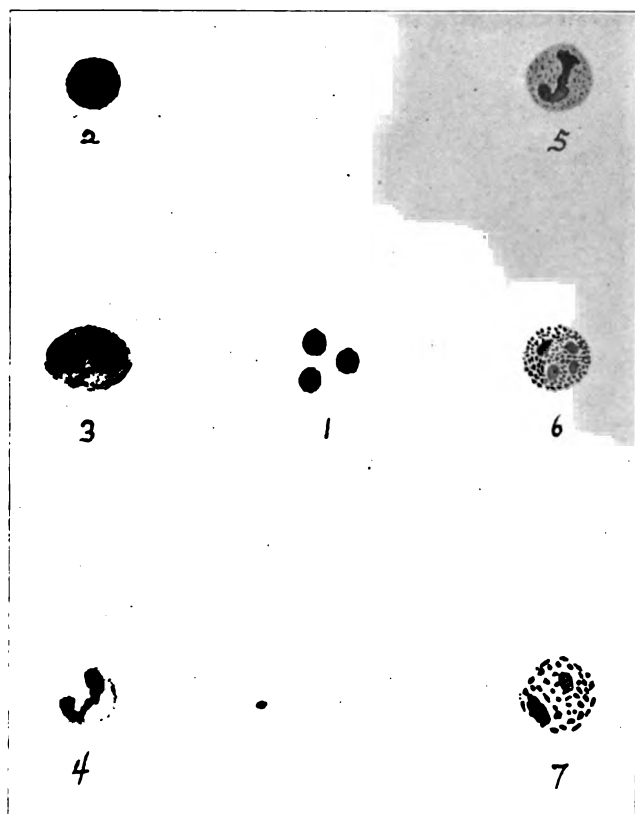
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FIG. 4.

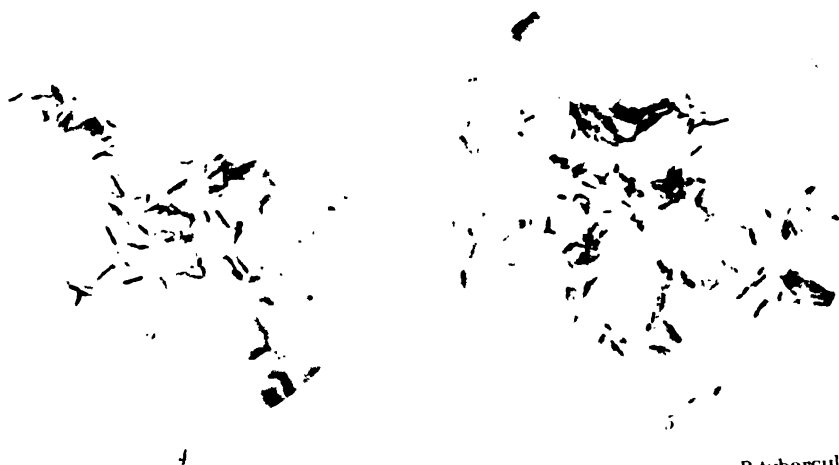
RAVENEL AND MCCARTHY.

FORAGE POISONING.



BUSCH AND VAN BERGEN.

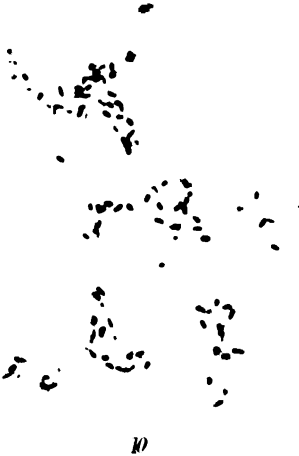
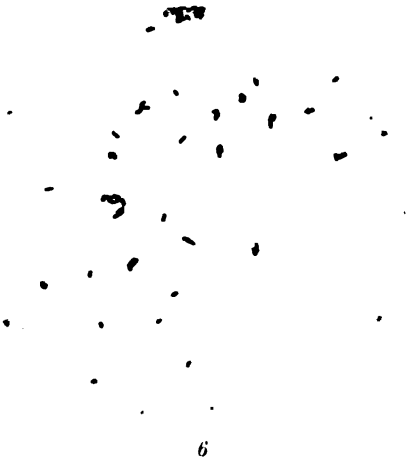
CAT'S BLOOD.



B. tuberculosis

Wolbach and Ernst.

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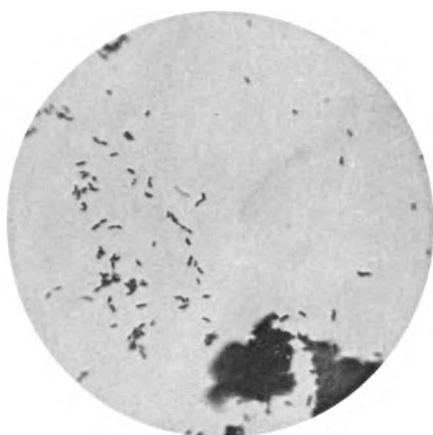


Wolbach and Ernst.

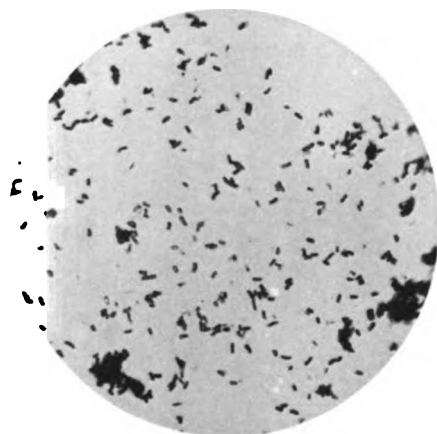
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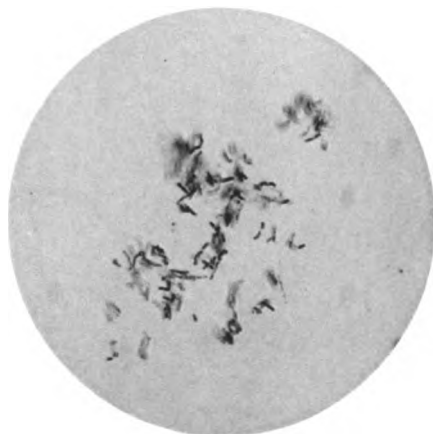
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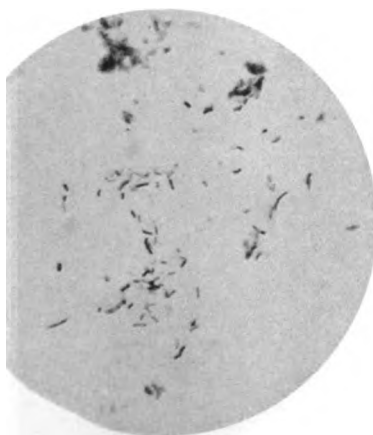
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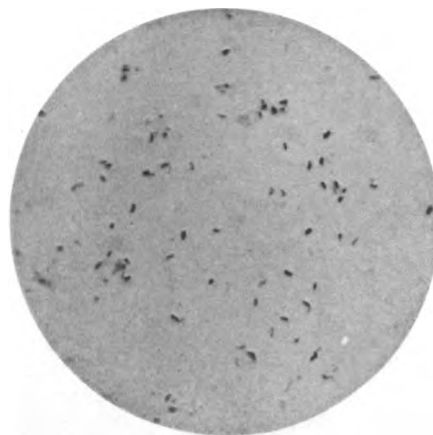
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Wolbach and Ernst.

B. tuberculosis



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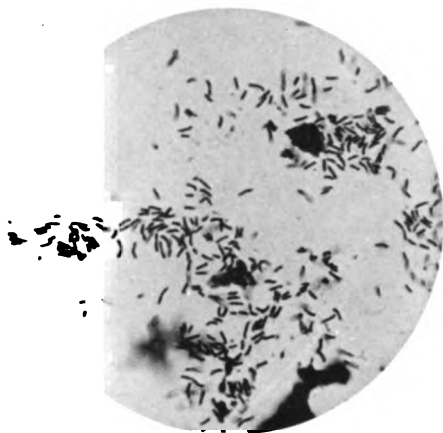
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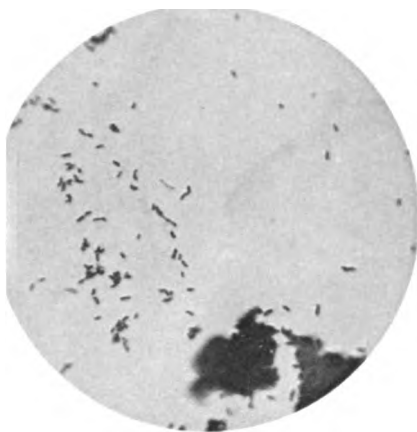
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Wolbach and Ernst.

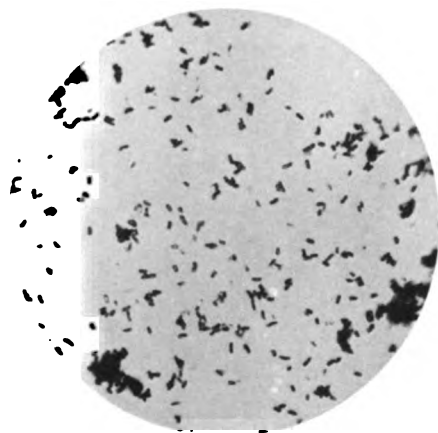
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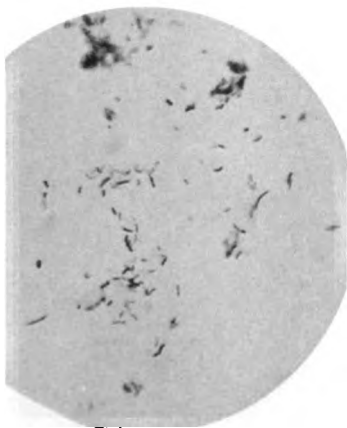
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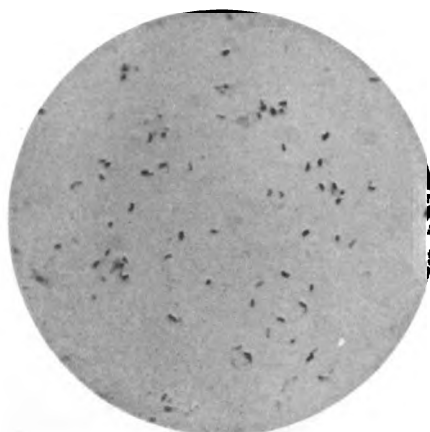
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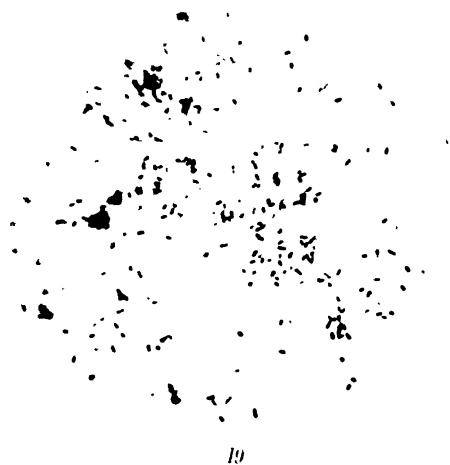
17

Wolbach and Ernst.

B. tuberculosis



18



19



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21



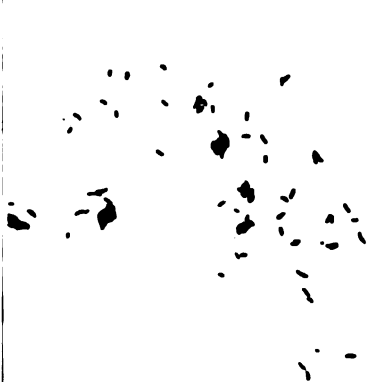
22



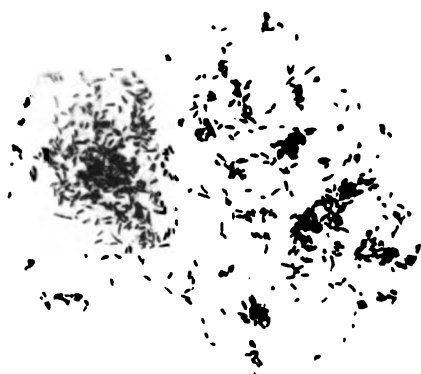
23

Wolbach and Ernst.

B.tuberculosis



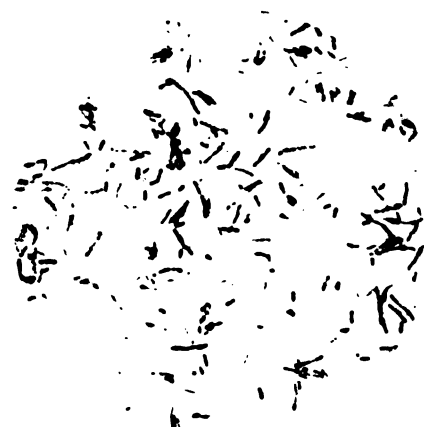
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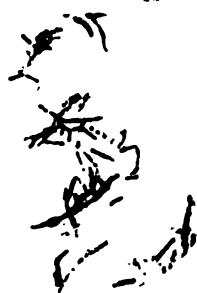
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26



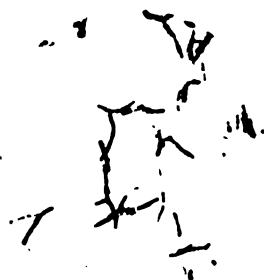
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31



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33

Wolbach and Ernst.

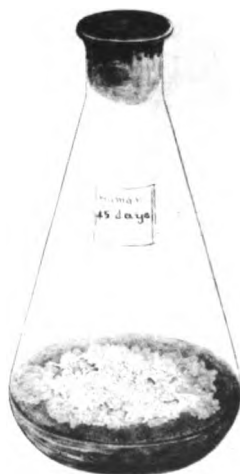
B. tuberculosis



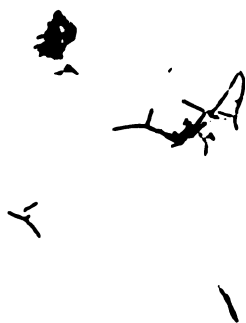
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35



36



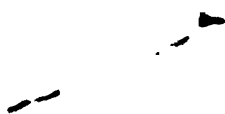
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38



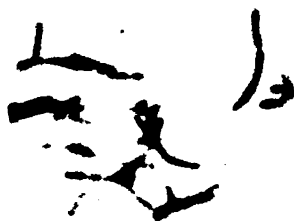
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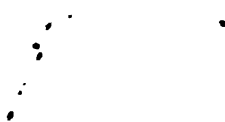
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41



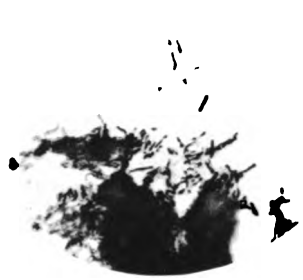
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43







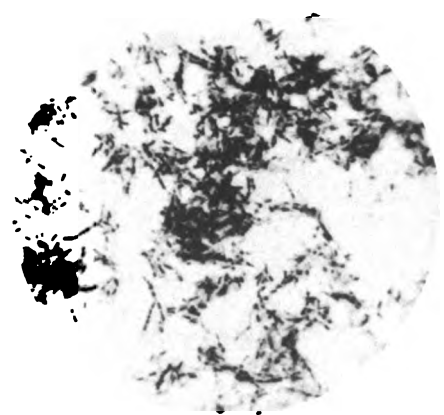
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53



54



55

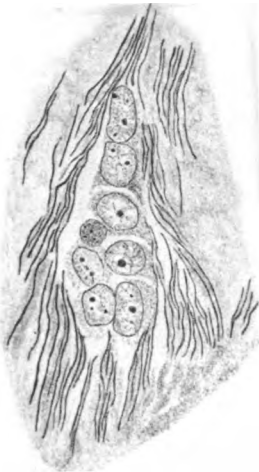
56

Wolbach and Ernst.

B. tuberculosis



1



2



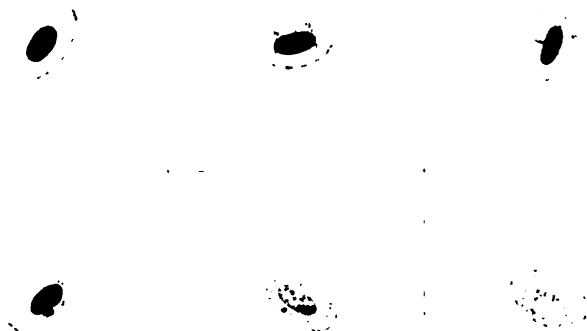
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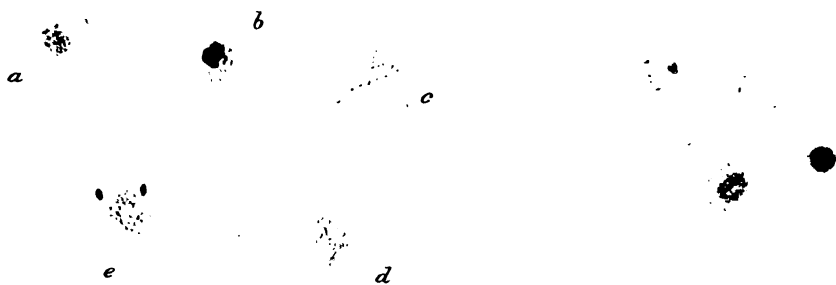
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Mallory.

Fibrillar substance.



1



2

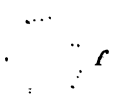
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4



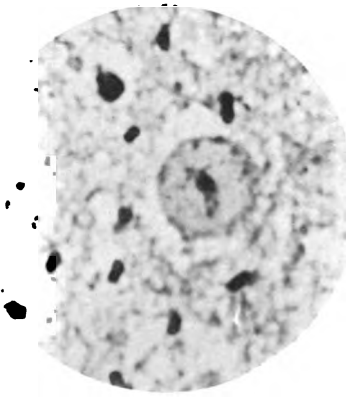
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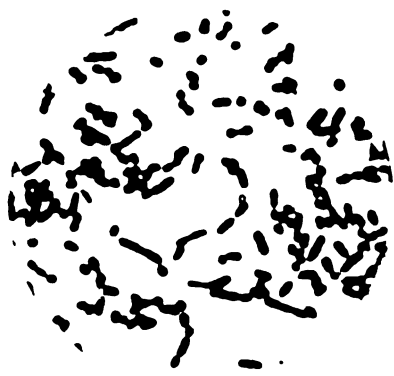
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6

5



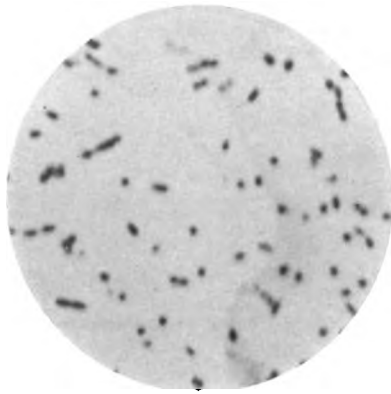
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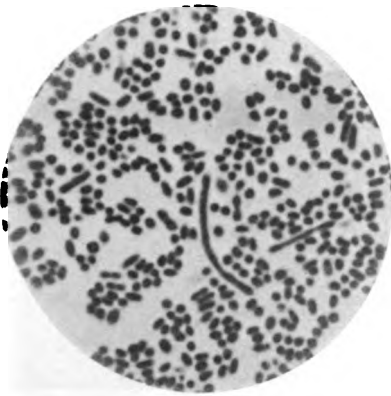
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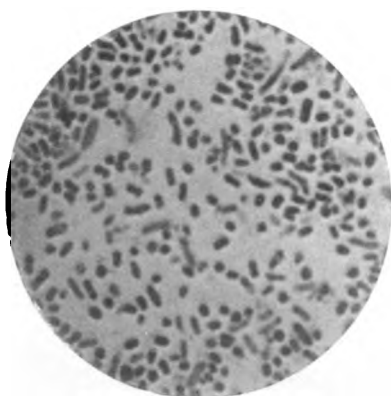
3



4



5



6

Berry and Ernst.

B. pyogenes sanguinarum



1

BROWN

Chinese foot.

H. W. Brown & Son



1

Brown

Chinese foot.

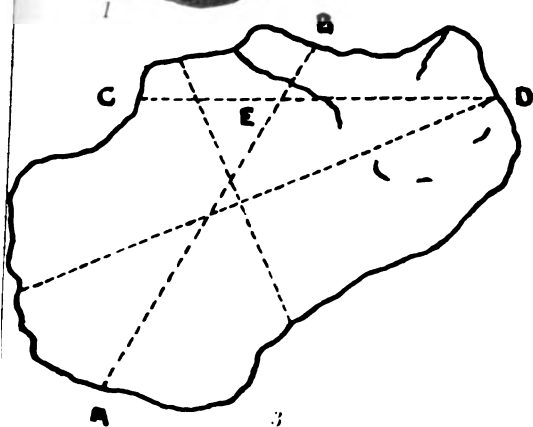
Helwig & Co., Berlin.



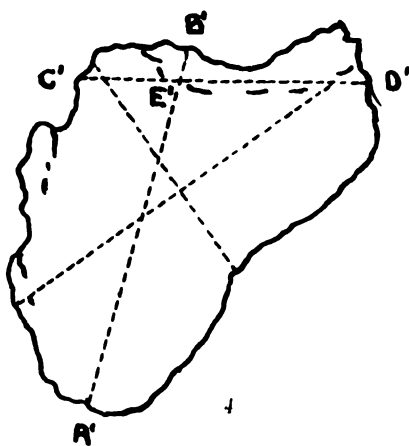
1



2



3



4



9



10

Brown

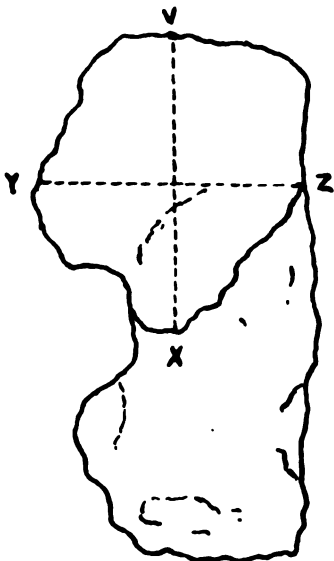
Chinese foot.



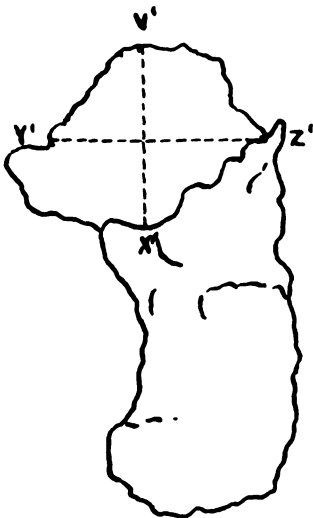
5



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7



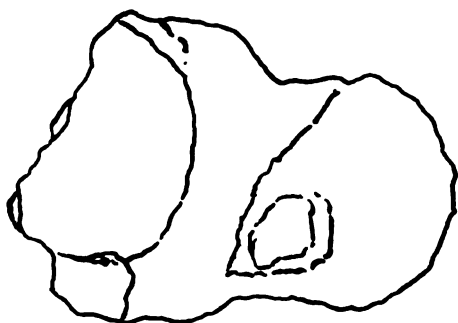
8

Brown

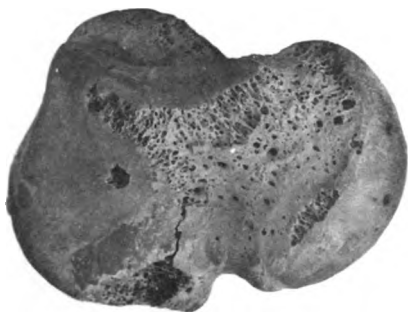
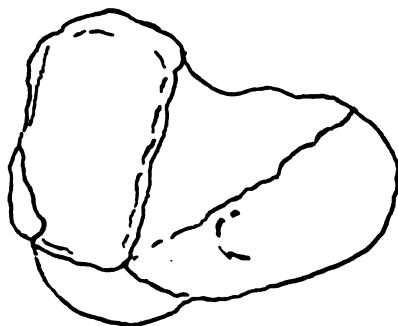
Chinese foot.



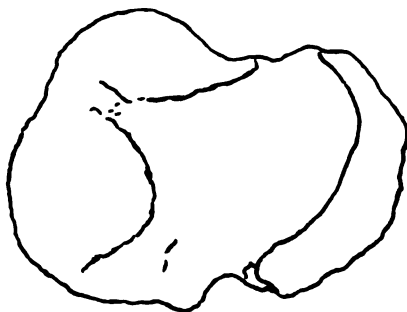
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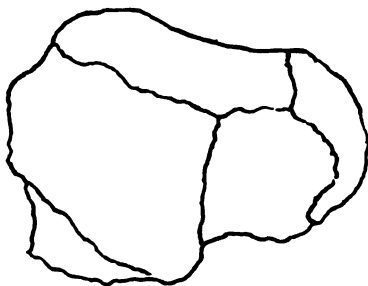
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3

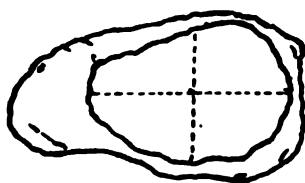
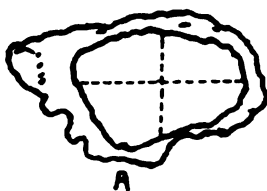


4



Brown

Chinese foot.



Brown

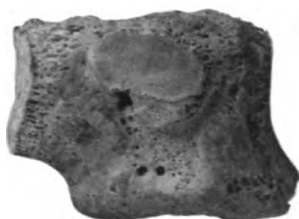
Chinese foot.



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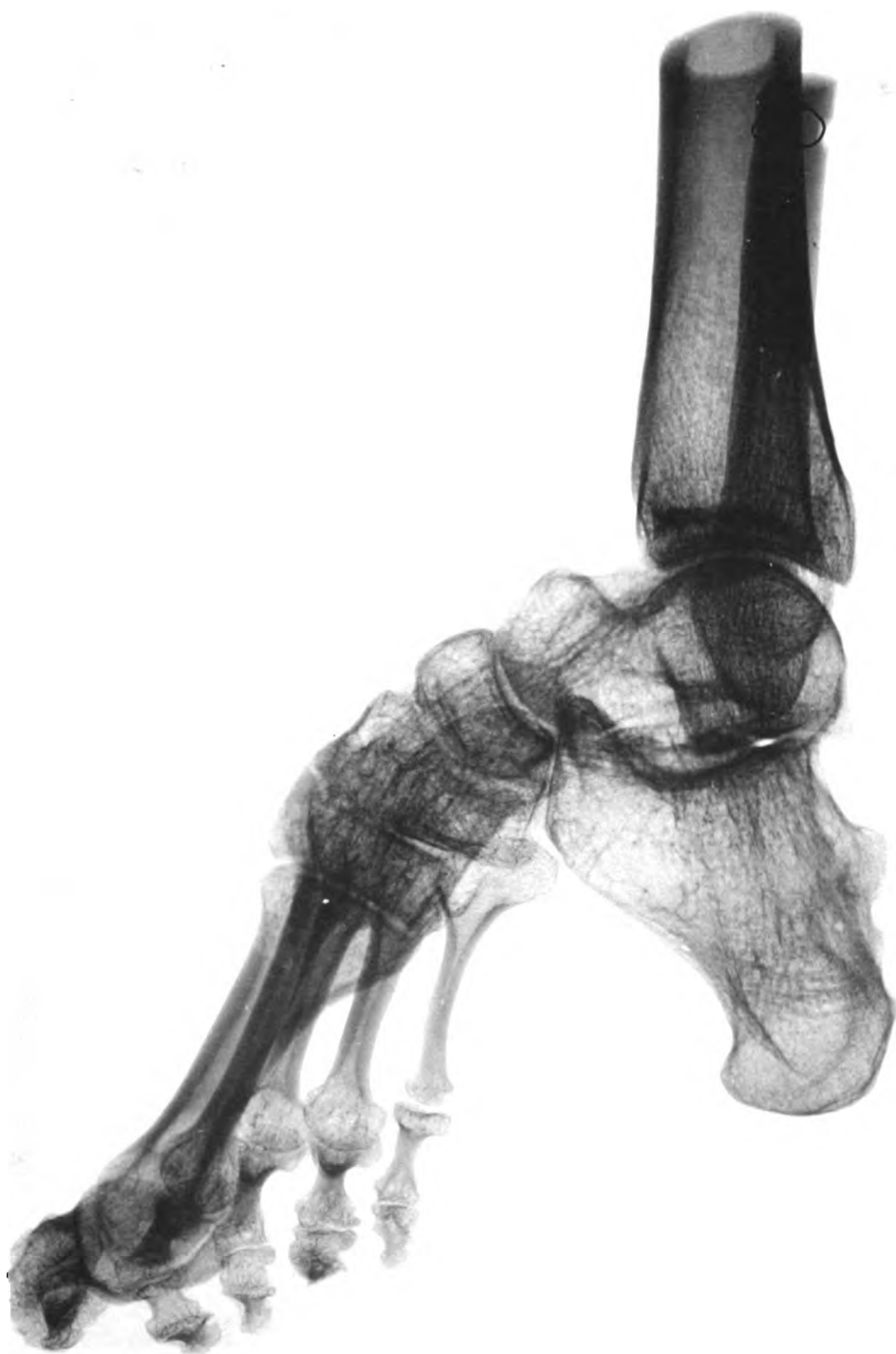
5



6

Brown

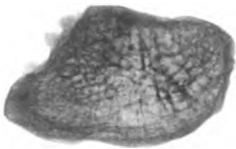
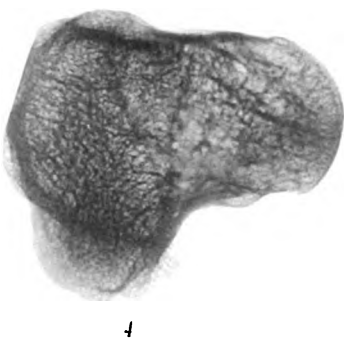
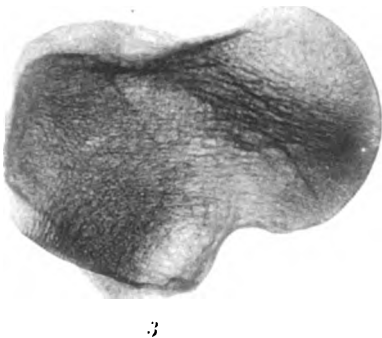
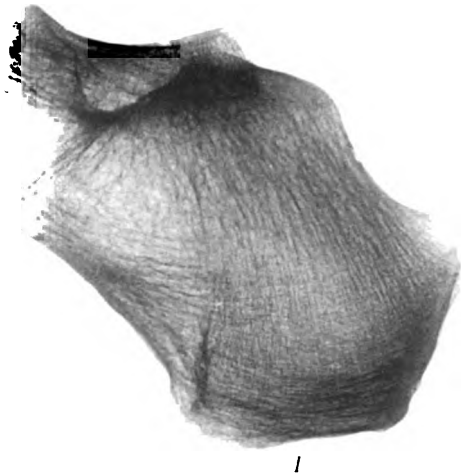
Chinese foot.



Brown

1

Chinese foot.

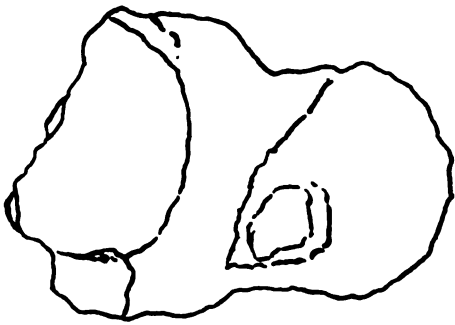


Brown

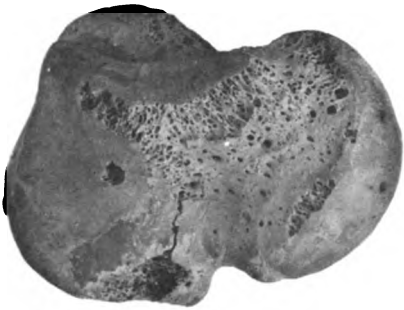
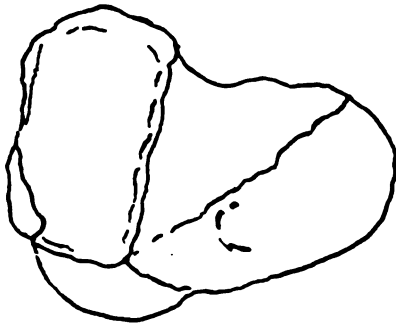
Chinese foot.



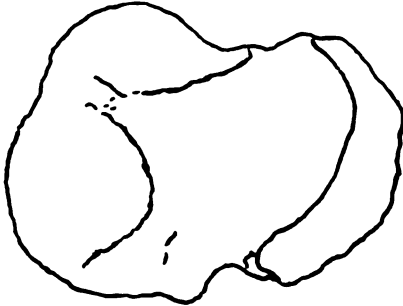
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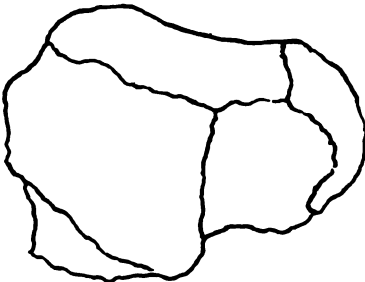
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4

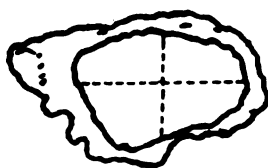


Brown

Chinese foot.



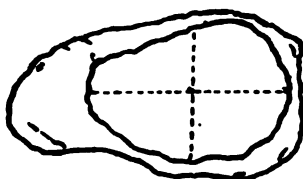
1



A



2



3



Brown

Chinese foot.



1



2



3



4



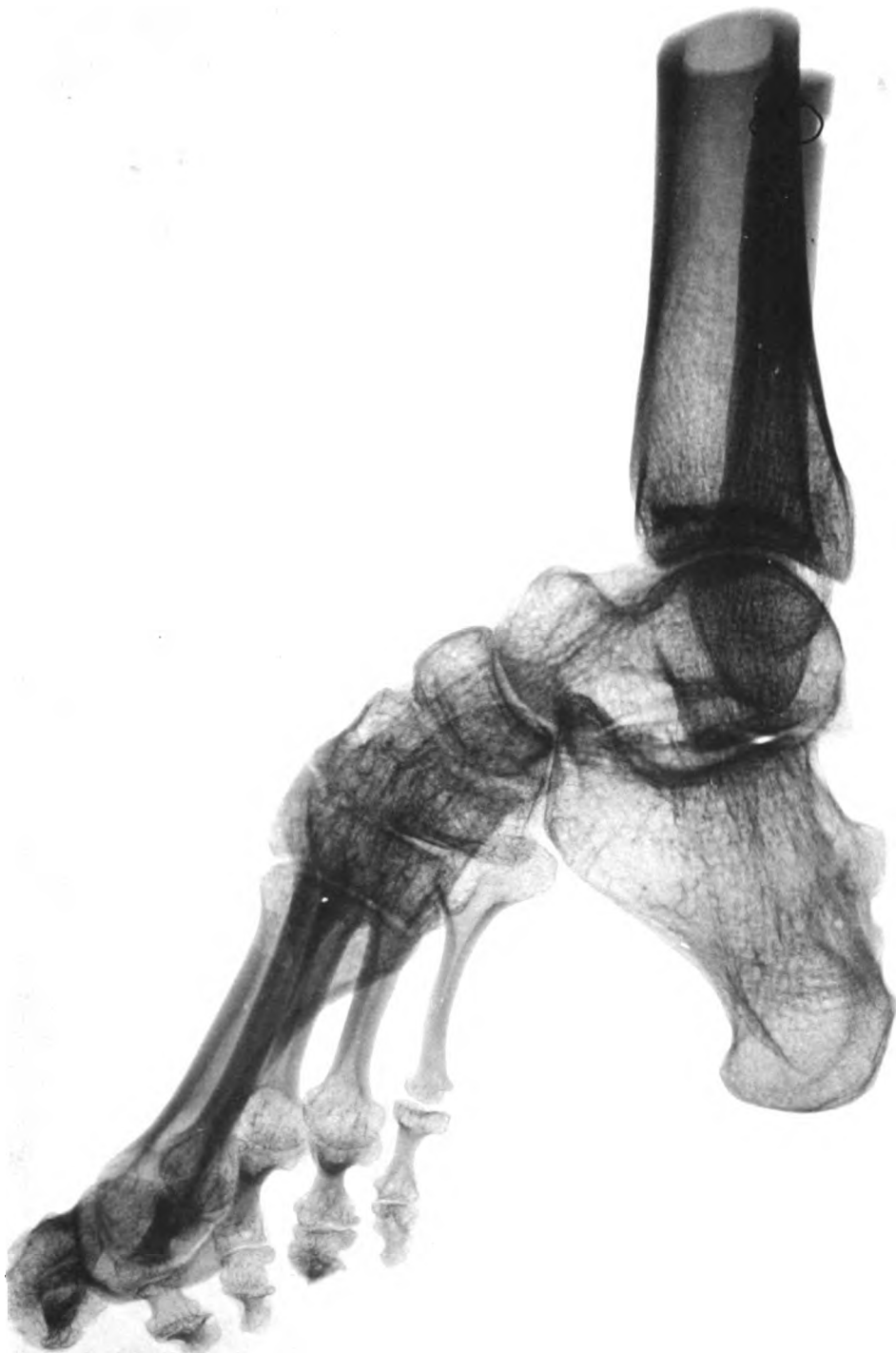
5



6

Brown

Chinese foot.



Brown

1

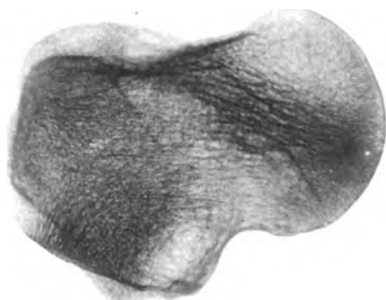
Chinese foot.



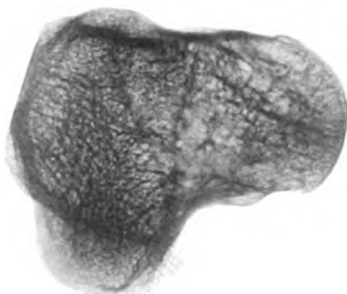
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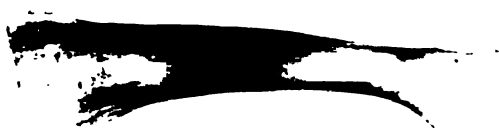
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3



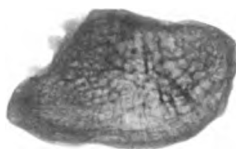
4



5



6



7



8

Brown

Chinese foot.

Fig. I

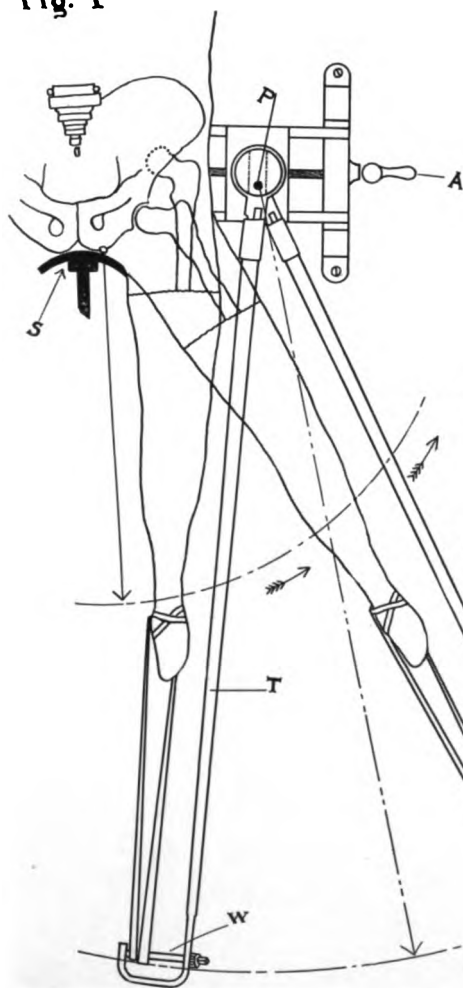


Fig. II

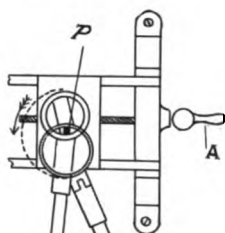


Fig. III

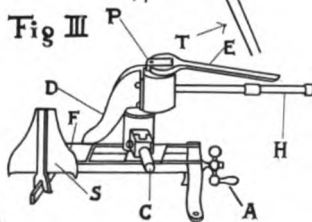
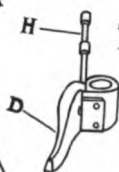


Fig. IV



BARTLETT.

CONGENITAL HIP.



1



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6

Brown

Chinese foot.



Brown

1

Chinese foot.



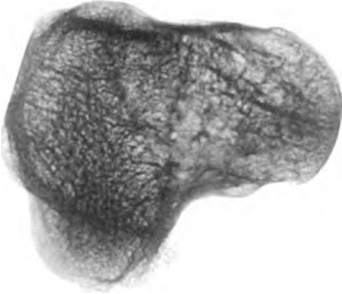
1



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3



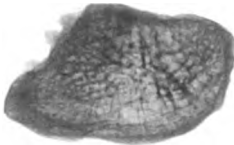
4



5



6



7



8

Brown

Chinese foot.

Fig. I

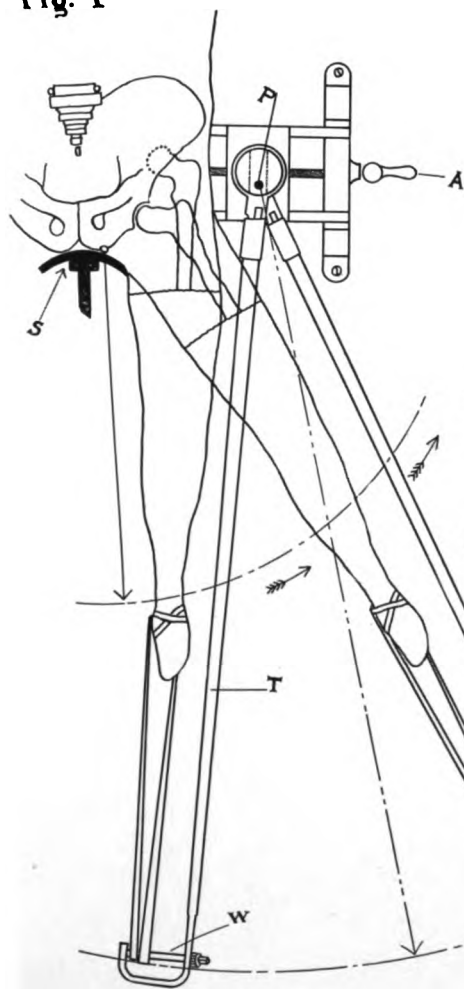


Fig. II

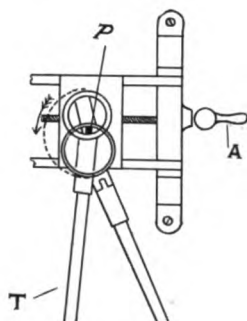


Fig. III

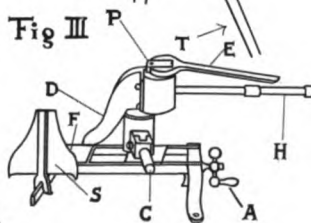
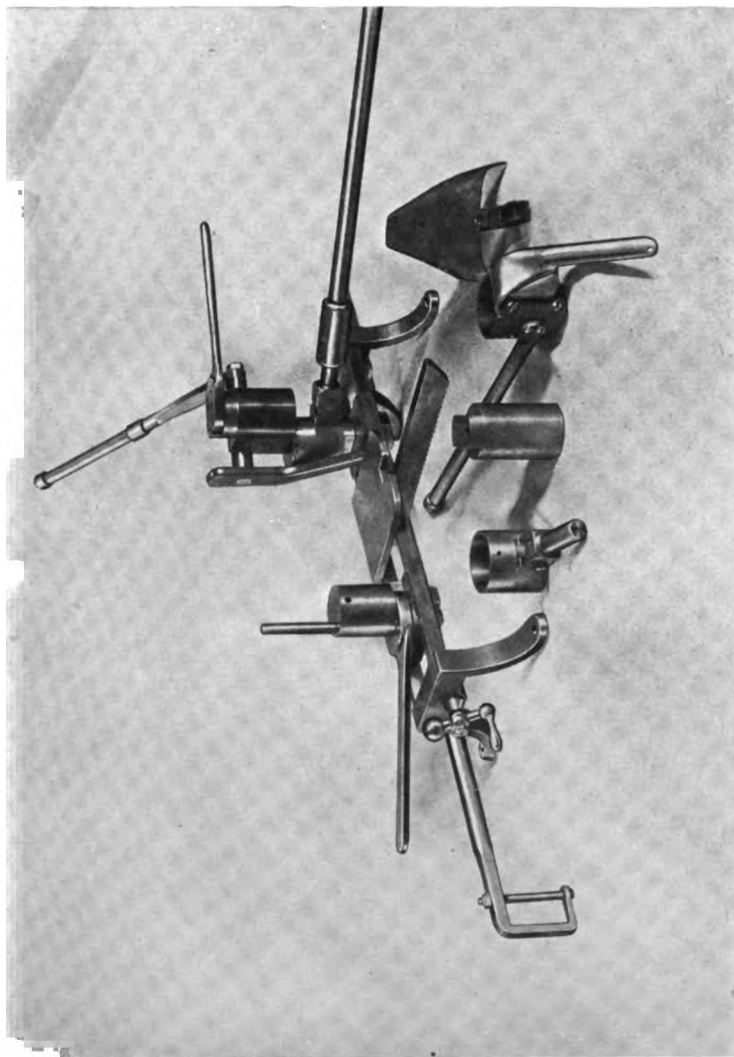


Fig. IV



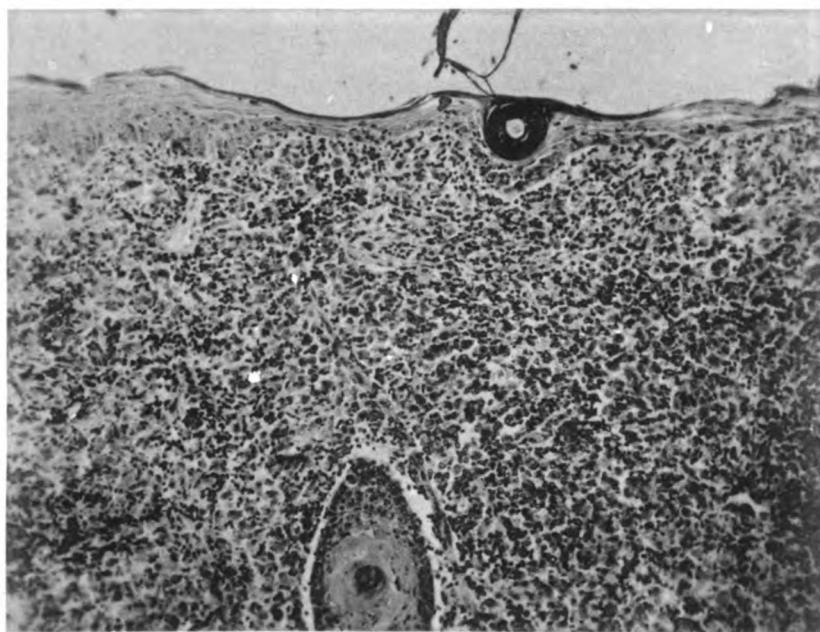
BARTLETT.

CONGENITAL HIP.

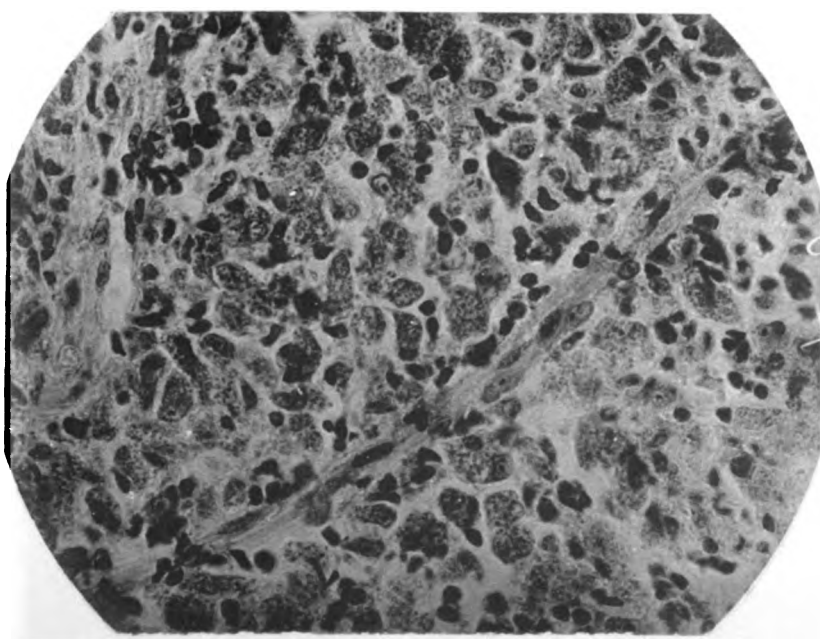


BARTLETT.

CONGENITAL HIP.



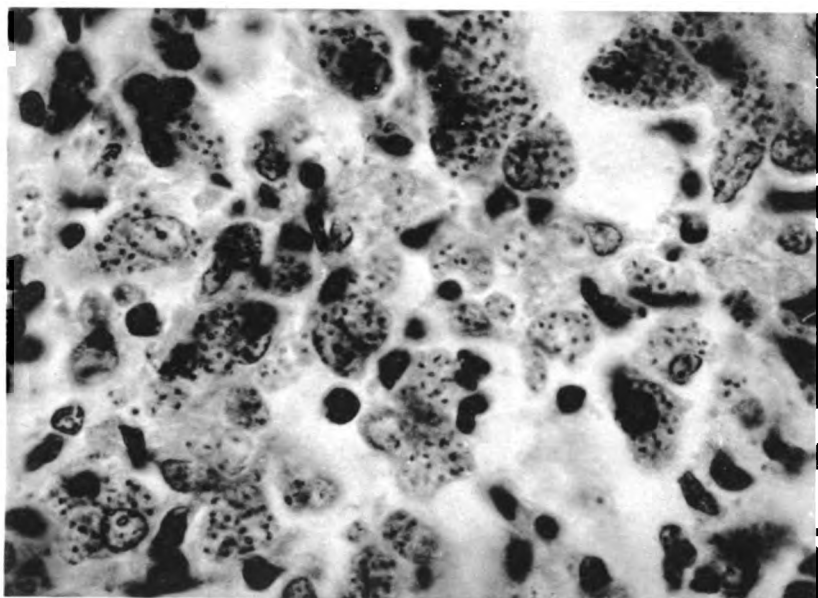
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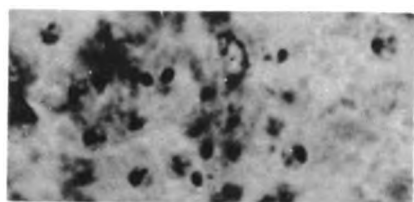
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Wright

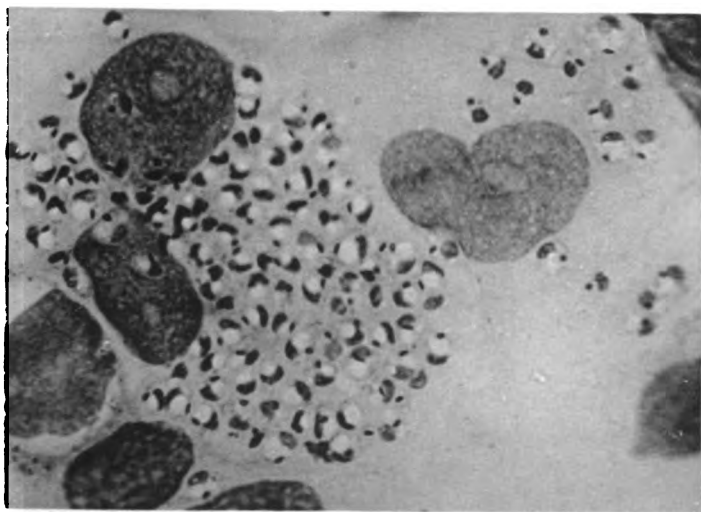
Tropical ulcer



3



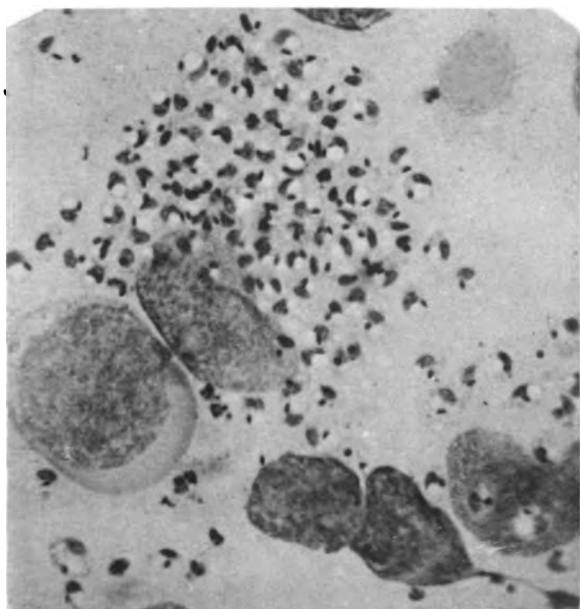
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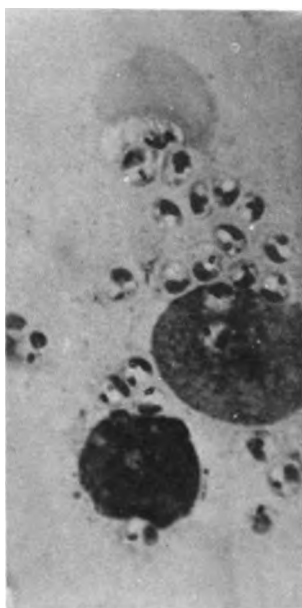
5

Wright

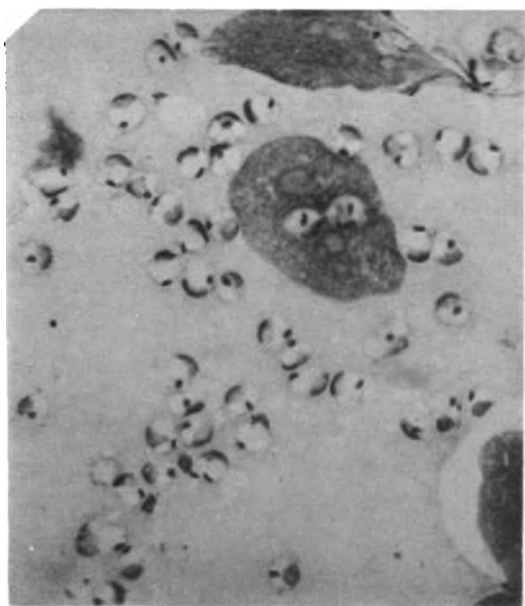
Tropical ulcer



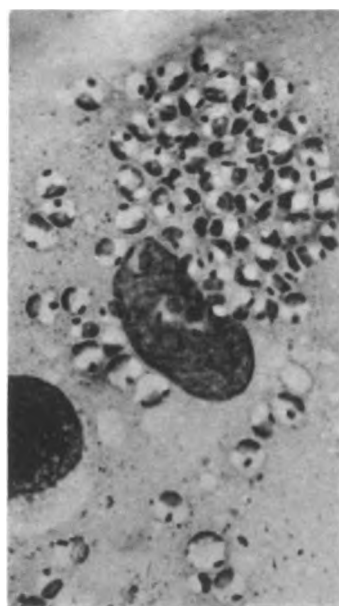
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7



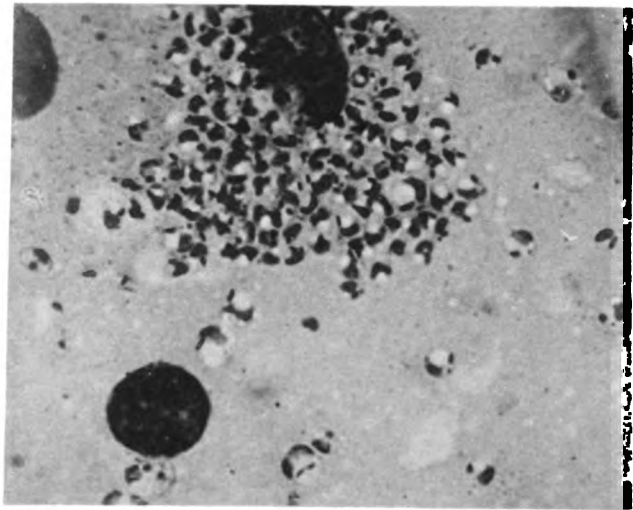
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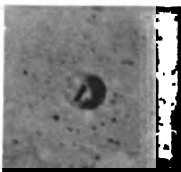
9

Wright

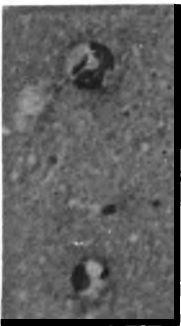
Tropical ulcer



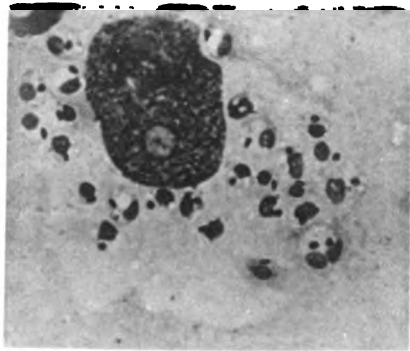
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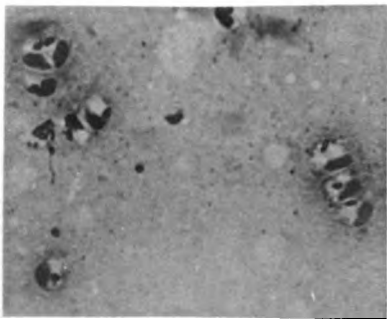
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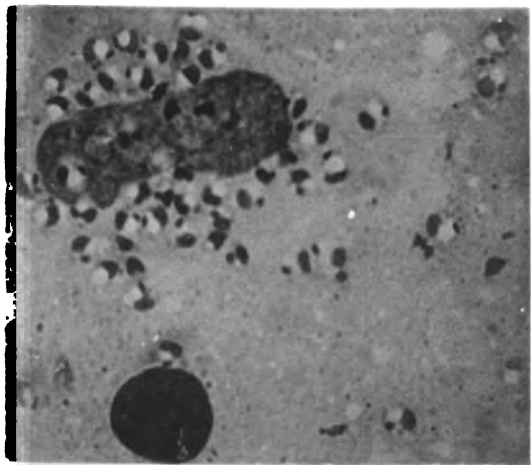
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14

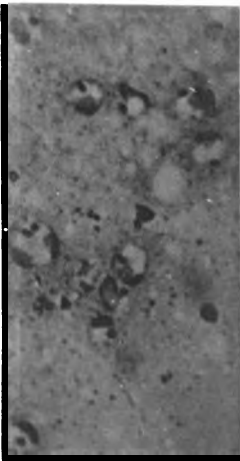


15



16

Wright



17

Tropical ulcer

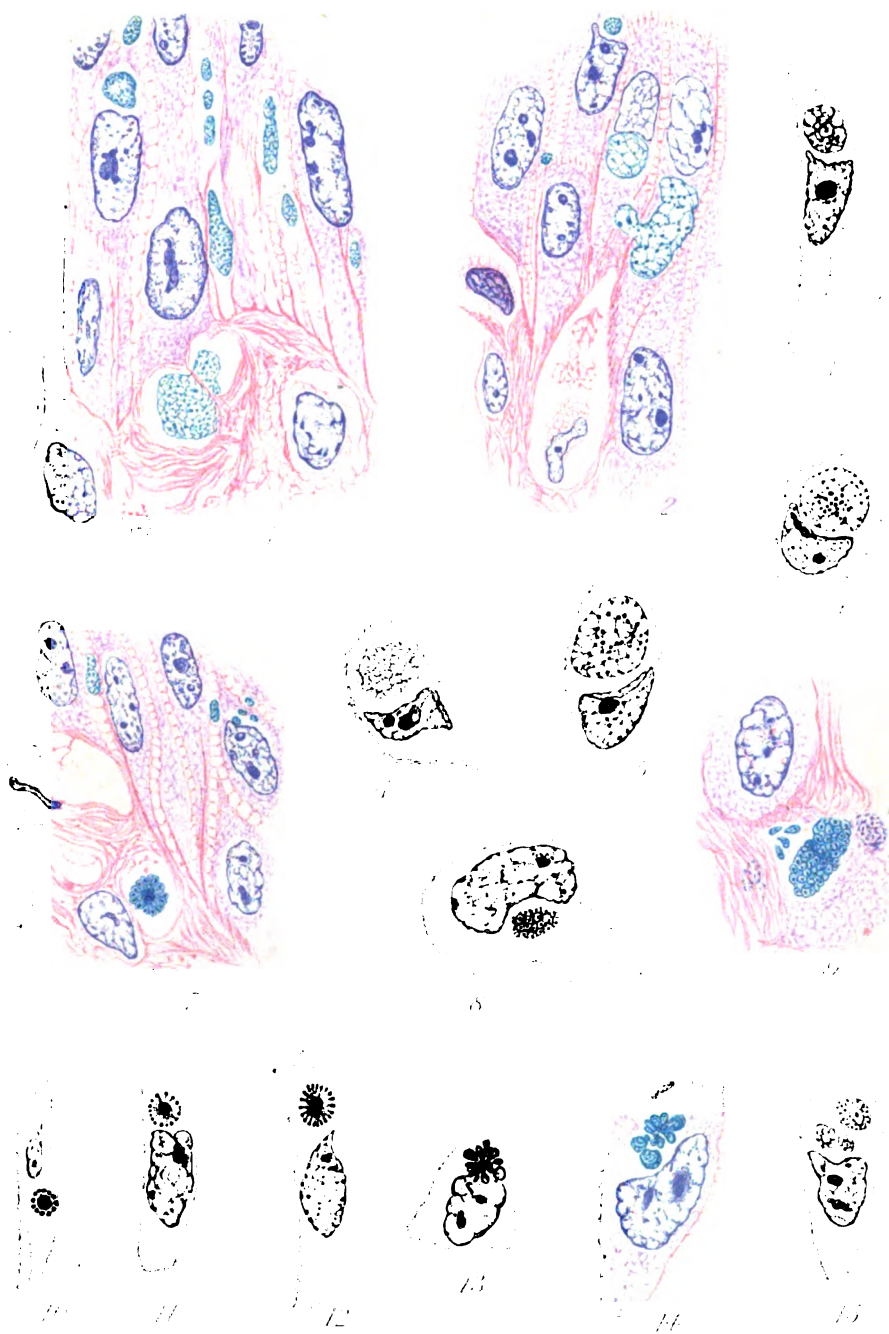
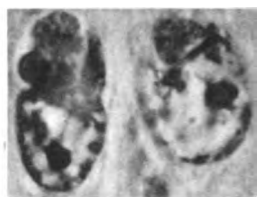


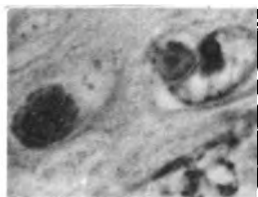
Fig. 1.

Fig. 2.

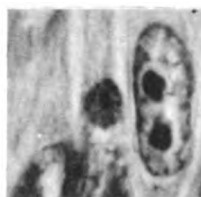
Fig. 3.



1



2



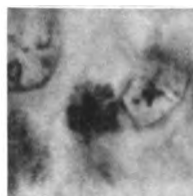
3



4



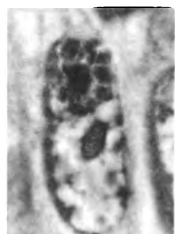
5



6



7



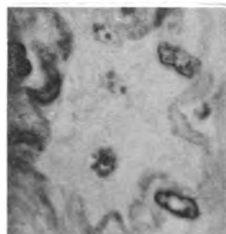
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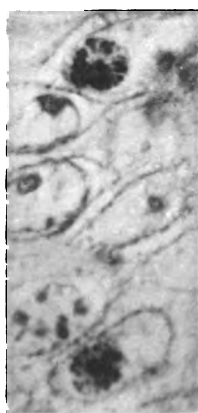
9



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3 2044 106 227 101

